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HISTOPHYSIOLOGICAL STUDIES ON THE JUVENILE ATLANTIC SALMON (SALMO SALAR) RETINA

I. RATES OF LIGHT- AND DARK-ADAPTATION[1]

M. A. ALI, W. R. STEVENSON, AND JUDITH S. PRESS

Abstract

The structure of the Atlantic salmon retina corresponds to descriptions by previous workers and is also similar to that of the Pacific salmon retina. Retinal epithelial pigment light-adapts in 60 minutes. Cones light-adapt in 45 minutes. Dark-adaptation of the pigment and cones occurs in 70 minutes. Retinal index shows light-adaptation occurring in 60 minutes and dark-adaptation in 70 minutes. Results are compared with those of the Pacific salmon's.

Introduction

The structure of the Atlantic salmon eye has been the subject of many studies (7, 8). However, as far as we know, no histophysiological studies of its retina have been made. This paper is the first of a series dealing with the histophysiology of the juvenile Atlantic salmon retina. The rates of light- and dark-adaptation of retinal elements have been studied using histological methods. An attempt has been made to describe the total rate of adaptation of the eye in a quantitative manner. These results are compared with those available for other salmonids (1), and their phylogenetic significance ascertained. It is hoped to correlate these results, later, with the behavioral responses of the fish such as was done in some previous investigations (1, 2, 4).

Material and Methods

Material

Atlantic salmon yearlings obtained from the Margaree Hatchery, Frizzleton, Nova Scotia, and kept in the Fish Research Laboratories of Memorial University were used in this investigation. These fish were kept in aerated and refrigerated running water (9–14° C). The amount of light entering the hatchery varied with the duration and intensity of natural light. The fish were fed daily on a diet of ground raw beef liver. Their lengths ranged from 5.3 cm to 8.5 cm.

[1]Manuscript received October 24, 1960.

Contribution from the Fish Research Laboratories, Memorial University of Newfoundland, St. John's, Newfoundland. This investigation was supported by the National Research Council of Canada through grants to M. A. Ali.

[The previous number of Can. J. Zool. (**39**, 1–122) was issued February 20, 1961.]

Methods

Experimental.—The experiments whose results are described in this paper were conducted during the first week of June, 1960. Fish were left in light (25 ft-c), or darkness, overnight (18 hours) and on the following morning five fish were sampled (0 minutes) before the experiment commenced. After this, the light was turned on (for light-adaptation) or was turned off (for dark-adaptation) and five fish each taken at the various intervals shown in the graphs (Figs. 3, 4).

Histological.—Fish were fixed in Bouin's fixative and their eyes punctured at the sclerocorneal junction in order to facilitate better fixation of the retina. After 48 hours in Bouin's the fish were transferred to 70% alcohol, measured, and weighed. Their eyes were then enucleated and the cornea and the lens removed. They were dehydrated in ascending grades of alcohol, cleared in xylene, and imbedded in paraffin (Fisher Tissuemat: 56–58° C m.p.). Sections were cut at 8 μ, stained with Harris' haematoxylin, and counterstained with eosin. Stained sections were mounted in Canada balsam and dried (37° C) for at least 24 hours before being examined.

Measurements.—The region between the choroid and the internal limiting membrane was considered the retina (Fig. 1). The retinal epithelial pigment was measured as the distance between the choroid and the extreme margin of the pigment within the epithelial cells (Fig. 2). Only the part distal to the external limiting membrane (Fig. 2) was measured in the case of the cones since only this region of the cone undergoes photomechanical changes (1). Measurements were made with a calibrated ocular micrometer. Five measurements from five regions of the retina were made. Each point in the graph represents the mean of 50 measurements from 10 eyes.

Calculation of retinal index.—Since the thicknesses of the retinal layers do not vary in direct proportion to the thickness of the retina (3), graphs have been constructed using direct thicknesses. However, since the responses of the pigment and cone layers to light and dark are opposed to one another, it was thought that an index which embraces both these thicknesses may provide the quantitative means by which the rates of adaptation of both layers could be described. The retinal index was calculated using the following formula:

$$\text{Retinal index} = P/(P + C)$$

where P = thickness of pigment layer,

C = thickness of cone layer.

Graphs wherein retinal index is plotted against time are also given (Figs. 3, 4) and appear to describe total adaptation well.

Results

General Observations

The retina of the Atlantic salmon consists of eight layers and two membranes (Fig. 1), which are listed below.

(1) Epithelial pigment layer
(2) Visual cell layer (rods and cones)
(3) External limiting membrane
(4) External nuclear layer
(5) External plexiform layer
(6) Internal nuclear layer
(7) Internal plexiform layer
(8) Ganglion cell layer
(9) Nerve fiber layer
(10) Internal limiting membrane

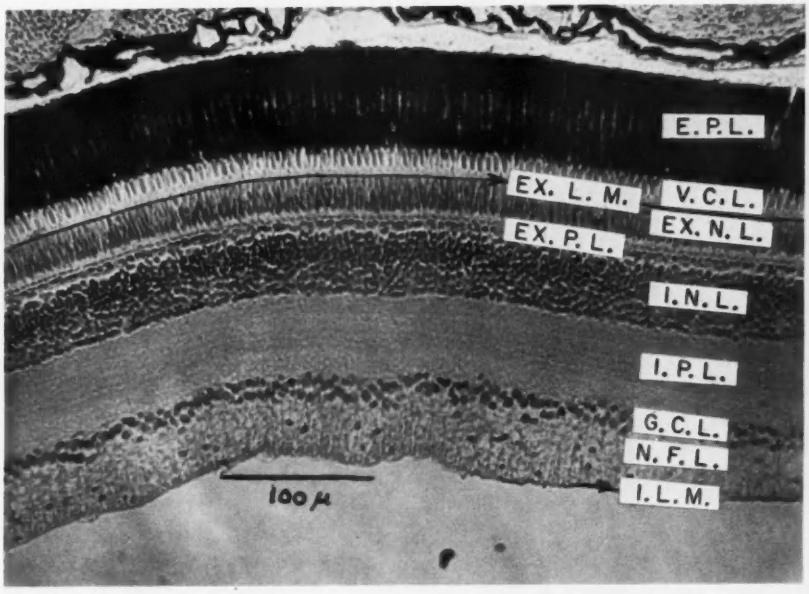

FIG. 1. Photomicrograph of a cross section of a light-adapted retina.

E.P.L.	Epithelial pigment layer.	I.N.L.	Internal nuclear layer.
V.C.L.	Visual cell layer (rods and cones).	I.P.L.	Internal plexiform layer.
EX.L.M.	External limiting membrane.	G.C.L.	Ganglion cell layer.
EX.N.L.	External nuclear layer.	N.F.L.	Nerve fiber layer.
EX.P.L.	External plexiform layer.	I.L.M.	Internal limiting membrane.

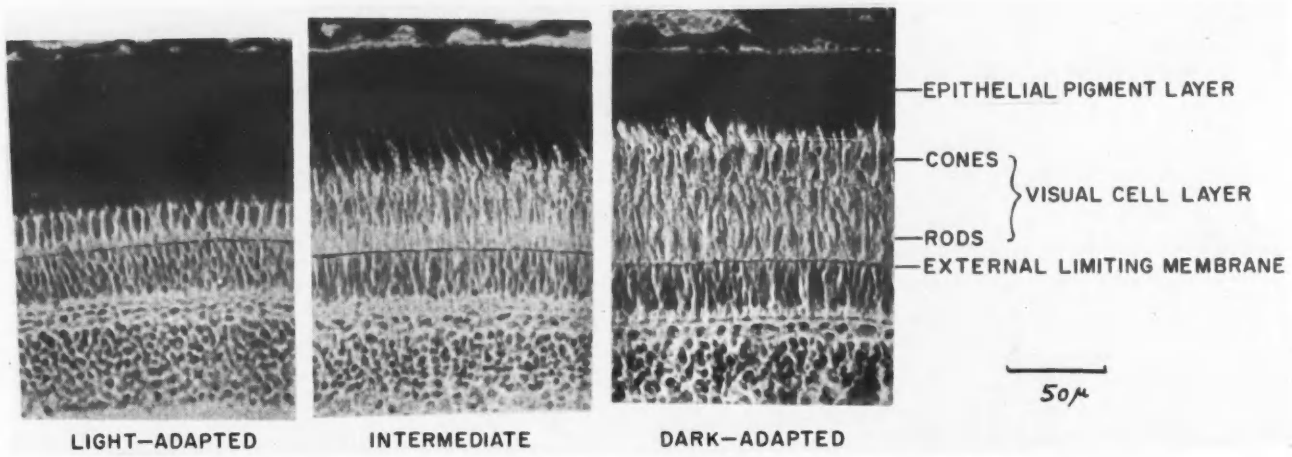

FIG. 2. Photomicrographs of cross sections of the retina in three different states of adaptation.

The structure of the retina, as observed in this study, corresponds to those described by previous workers (7, 8). It is also similar to that of the Pacific salmon retina (1) in its appearance and structure.

No difference in the staining reactions of any of the retinal layers is observed between the light- and dark-adapted retinae. The only changes observed were photomechanical (Fig. 2). In light, the retinal epithelial pigment is fully expanded (or dispersed) and extends well into the tips of the epithelial cell protuberances. The cones lie close to the external limiting membrane as a result of the contraction of the myoids. The rods, which are expanded due to the relaxation of their myoids, are masked by the expanded pigment and cannot be seen clearly. In the dark-adapted state, the pigment is fully contracted and has the appearance of a dark, narrow strip. The cones are fully expanded with their outer segments almost touching the pigment. The rods lie adjacent to the external limiting membrane and can be seen quite clearly (Fig. 2).

Light-adaptation (Fig. 3)

The response of both the pigment and the cones to light is immediate and proceeds at a steady pace. However, the pigment attains a fully light-adapted

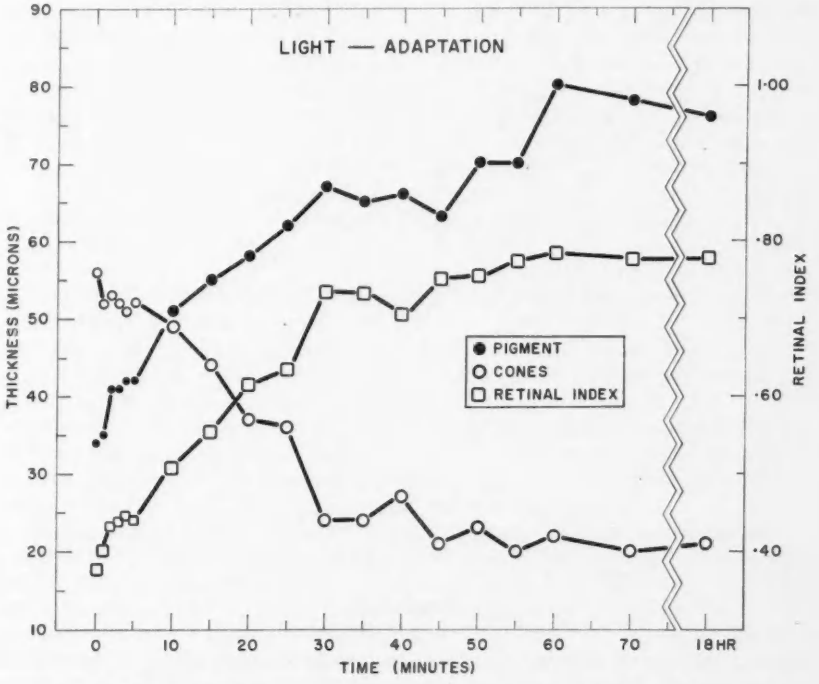

FIG. 3. Graph showing the rates of adaptation of retinal epithelial pigment and cone layers when the dark-adapted fish were exposed to light and sampled at the intervals shown. Retinal indices are also shown.

state only 60 minutes after exposure to light while the cones become fully contracted (light-adapted) 45 minutes after exposure to light.

When the retinal index is plotted against time it is seen that total adaptation of both pigment and cones occurs 60 minutes after exposure to light. This curve is much smoother and may very well be considered as showing the adaptation rate of the entire retina.

Dark-adaptation (Fig. 4)

No latent period before the commencement of migration of either the pigment or cones due to the exposure to dark is seen. In both cases the response is almost a linear one and full dark-adaptation occurs 70 minutes after exposure to dark.

The graph plotted using the retinal index confirms the above observations. Here the response is almost perfectly linear.

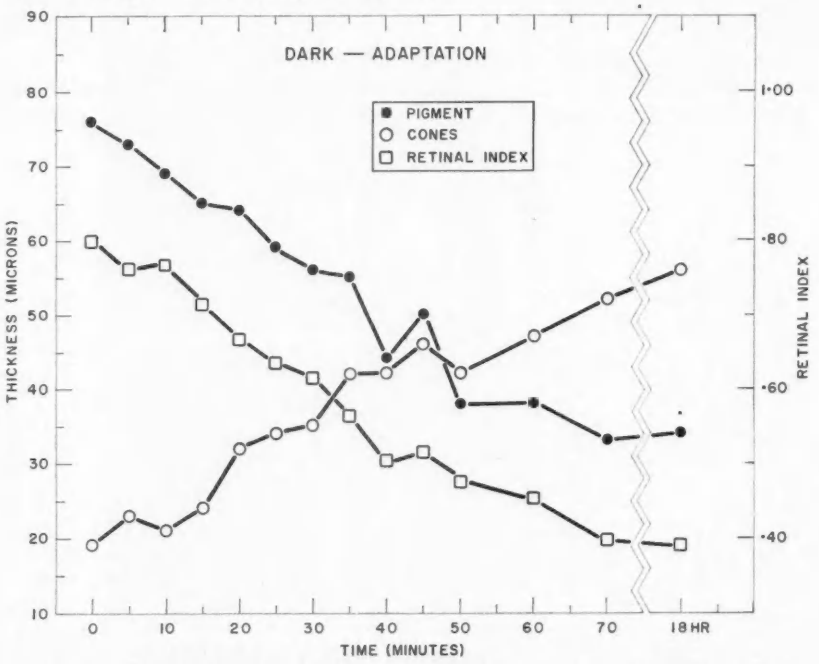

FIG. 4. Rates of adaptation of retinal epithelial pigment and cone layers when light-adapted fish were subjected to darkness and sampled at the intervals shown. Retinal indices are also plotted.

Discussion

Both the times for light- and dark-adaptation are longer in the Atlantic salmon yearling than in any of the stages of the four species of Pacific salmon (genus *Oncorhynchus*) that have been studied (1). Among the Pacific salmon, the spring (*O. tshawytscha*) and coho (*O. kisutch*) are related on the one hand,

the pink (*O. gorbuscha*) and chum (*O. keta*) on the other, while the sockeye occupies an intermediate position (5). Ethologically, the coho is suggested to be closest to the parental, trout-like type, while the pink and chum are the most specialized (6). In view of this, the Atlantic salmon should be more closely related to the coho and spring than it is to the other members of the genus *Oncorhynchus*. Indeed in its migratory and territorial behavior, the Atlantic salmon is similar to the coho and is very different from the pink or chum, which migrate earlier and possess schooling behavior. However, the results of this study do not show any similarites.

While bearing in mind the differences between the Atlantic salmon yearling and the various species and stages of the juvenile Pacific salmon, in so far as their life histories and ages are concerned, it is interesting to compare their rates of adaptation. The pigment of the Atlantic salmon has taken 60 minutes to light-adapt as compared to the 25 minutes (the longest) taken by the pigment of sockeye and pink emerged fry (1). The pigment of the three stages of coho studied took only 15–20 minutes to light-adapt. The cones of the Atlantic salmon have also taken considerably longer time (45 minutes) to light-adapt than those of coho or pink emerged fry, which took 25 minutes.

In the case of dark-adaptation also the retinal elements of the Atlantic salmon yearling have taken a much longer time, 70 minutes as compared to the 40 minutes taken by the pigment of sockeye and coho smolts and pink emerged fry, and 50 minutes taken by the cones of the sockeye smolt. In addition, the retinal pigment of the Atlantic salmon did not show a latent period prior to contraction in the dark. In this respect it shows quite a difference from the various stages of the sockeye, coho, and chum and with the emerged fry stage of the pink. However, it is interesting to note that the pigment of the late fry stage of pink, like the pigment of the Atlantic salmon yearling, did not possess a latent period during dark-adaptation.

In a general way, the similarities between the Atlantic salmon and the Pacific salmon, in so far as adaptation periods are concerned, are (i) light-adaptation is faster than dark-adaptation and (ii) the cones generally respond more quickly than the pigment.

Thus, it may be concluded that the Atlantic salmon yearling's rates of retinal adaptation show more differences than similarities with those of the Pacific salmon's.

A possible reason for the differences between the rates of adaptation in the Atlantic salmon and in the Pacific salmon may be the use of a lower light intensity (25 ft-c) in the experiments with the former while in those with the latter a much higher light intensity (400 ft-c) was employed.

In the case of light-adaptation, it would appear that an animal which is subjected to a brighter light after a period in total darkness will light-adapt faster than an animal which is subjected to a lower light intensity. This may be attributed to the fact that light is an active stimulus and the higher its intensity the greater will be its stimulating effect.

Conversely, in the case of dark-adaptation, an animal that has been subjected to a higher light intensity before it is placed in darkness will be influenced by this light for a longer period than an animal which has been subjected to a

much lower intensity. This may explain the presence of a latent period in the case of most of the species and stages of Pacific salmon and its absence in the case of the Atlantic salmon yearling. It may further be postulated that the retina of the animal transferred from a bright area to darkness, will, after the initial latent period, tend to adapt to darkness a little more quickly due to the magnitude of the difference between the two conditions. On the other hand, the retina of the animal transferred to darkness from a much lower light intensity will tend to dark-adapt slowly and at an even pace (without any latent period) because of the difference between the two conditions being much smaller. Further investigations will be conducted to study these aspects in detail.

The use of the retinal index appears satisfactory in so far as the construction of graphs is concerned (Figs. 3, 4). However, the validity of these rates will be checked by comparing these figures with results of behavioral experiments wherein feeding and pseudorheotropic responses will be used as indices.

Acknowledgments

Our thanks are due to Mr. J. M. Butler and Mr. A. J. Baxter, Fish Culture Branch, Canada Department of Fisheries, for supplying the fish used in this investigation. To Dr. W. A. Clemens and to the reviewer of this manuscript we are obliged for their constructive criticisms.

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THE EFFECT OF CERTAIN CHEMICALS ON THE RESPONSE TO ELECTRICAL STIMULATION AND THE SPONTANEOUS RHYTHMICAL ACTIVITY OF LARVAE OF PHOCANEMA DECIPIENS[1]

CLARE BRADLEY

Abstract

The effect of six neuromimetic compounds on the frequency of spontaneous rhythmical contraction, on the maintenance of tonic contraction, and on the response to anodal and cathodal stimulation was tested on *Phocanema* muscle preparations. Their effects could not be related to their hyper- or de-polarizing action. They had different effects on rhythmical activity, tonic contraction, and the response to electrical stimulation and this showed that these movements must be produced by independent effector systems.

Introduction

The experiments described in this paper are part of an investigation of the rhythmical movements of nematodes and the factors which control these movements. The response of two nematode muscle preparations to anodal and cathodal electrical stimulation was described previously (1, 2). It was shown that the response made by a preparation varies with the polarity of the stimulus and that an anodal stimulus elicits contraction and a cathodal stimulus relaxation.

It is interesting to compare the responses to electrical stimulation with the effect of various compounds which are known to have a depolarizing or hyperpolarizing action on nerve–muscle preparations of other animals. Among the compounds which were tested in the present series of experiments were nicotine, succinyl choline, and D-tubocurarine, which are depolarizing agents (the last two acting competitively with acetyl choline) and gamma-amino-butyric acid (GABA), which causes the hyperpolarization (or repolarization as it is sometimes called) of cell membranes.

Norton and de Beer (8) showed that succinyl choline and nicotine produce contraction of an *Ascaris* nerve–muscle preparation, whereas D-tubocurarine has no effect. The effect of GABA has not previously been tested on nematode material. Its action on a crustacean preparation was found to be similar to that produced by stimulation of the inhibitory nerve supply, and Elliott and Jasper (4) suggested that GABA is the transmitter substance in the crustacean inhibitory axons. Cathodal stimulation results in the relaxation of a *Phocanema* muscle preparation; a study of the change in this response at near freezing temperatures suggested that the stimulus was transmitted to the muscle cells along some nerve pathway, which would, by definition, be known as an inhibitory nerve (2). Because of the possibility that there is an inhibitory nerve supply to nematode muscle cells, it was thought that it would be interesting to make some observations on the action of GABA on these cells.

[1]Manuscript received December 1, 1960.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

For the same reason, the effect of 5-hydroxytryptamine (serotonin) was also tested. Hoyle (6) showed that this compound caused the relaxation of the anterior byssus retractor muscle of the lamellibranch *Mytilus* and suggested that serotonin is related to the transmitter substance at the inhibitory nerve endings of synapses in molluscs. Cells, such as the mammalian chromaffin cells, which contain serotonin, stain heavily with silver. There are chains of cells in the hypodermis of *Ascaris* which stain heavily with silver; Bradley (1) suggested that these cells may form some sort of nerve network with the possible function of transmitting inhibitory impulses to the muscle cells of the body wall, with serotonin acting as a transmitter substance. Mansour (7) has shown that serotonin stimulates the rhythmical activity of the liver fluke, *Fasciola hepatica*.

Finally, the effect of piperazine was tested, for Norton and de Beer (8) showed that this compound produced a flaccid paralysis of *Ascaris*.

Materials and Methods

Second-stage larvae of the nematode *Phocanema decipiens* were used in all the experiments. The method of storing the larvae and of setting up the muscle preparation, the recording apparatus, and the source of stimulation have already been described (2).

Two groups of experiments were planned, firstly to test the effect of various compounds on the responses to electrical stimulation, and secondly to observe their effect on the spontaneous rhythmical movements of *Phocanema*.

1. The Response to Electrical Stimulation

The Design of the Experiment

The experiment was planned as a crossover design (5) to test the effect of three compounds on the response of a preparation to a standard anodal stimulus. This design was chosen because there are considerable differences in the behavior of different worms, and it is therefore desirable to test the effect of all three compounds on each individual. This design has the additional advantage of increasing the number of degrees of freedom available for error control. The results of an experiment based on this design can be analyzed to show whether there are any residual effects of the three different treatments carried over into subsequent periods.

The three compounds tested are referred to as A, B, and C.

$$A = 1 \times 10^{-3} \text{ solution of GABA,}$$
$$B = 1 \times 10^{-4} \text{ solution of nicotine,}$$
$$C = 1 \times 10^{-3} \text{ solution of serotonin.}$$

Solutions A, B, and C were made up in Baldwin's medium. The sequence of treatments was arranged as follows:

	Columns (worms)					
Periods	1	2	3	4	5	6
1	A	A	B	B	C	C
2	B	C	A	C	A	B
3	C	B	C	A	B	A

The following schedule was used in each experiment:

The movements were recorded continuously from 30 to 150 minutes

Time	
0 minutes	Preparation set up (Baldwin's medium)
0– 30 minutes	Recovery period. No recording made
30– 40 minutes	Non-treatment period (Baldwin's medium)
40– 70 minutes	Treatment period No. 1 (A, B, or C)
70– 80 minutes	Non-treatment period (Baldwin's medium)
80–110 minutes	Treatment period No. 2 (A, B, or C)
110–120 minutes	Non-treatment period (Baldwin's medium)
120–150 minutes	Treatment period No. 3 (A, B, or C)

The particular sequence of treatments to be followed was assigned at random to the different worms. Two replicates of the experiment were done.

This experiment tested the action of GABA, nicotine, and serotonin on the response of a *Phocanema* nerve–muscle preparation to electrical stimulation. Some observations were also made on the effect of succinyl choline, piperazine, and D-tubocurarine on the response to anodal stimulation, and the effect of all six drugs on the response to cathodal stimulation. These observations, however, were not planned according to any particular statistical procedure. A recording was made of the response of the worm to stimulation before the drug was added to the muscle bath; the particular drug was then added, and the worm was again stimulated. The test solution was then replaced by Baldwin's medium and the stimulus repeated.

2. The Effect on Spontaneous Rhythmical Activity

A continuous recording was made of the spontaneous movements of each preparation under the influence of various compounds to be tested. Two measurements were made in each experiment. The first of these gave a measure of the level of tonic contraction maintained by the preparation. An inactive preparation usually maintained a steady base-line upon which all spontaneous movements were superimposed. Thus, at the end of each spontaneous contraction, the writing pen returned to the same position on the recorder chart, representing a return to a particular tonus level. The recorder chart was calibrated so that the base-line position could be read as a number on a scale. This allowed a measurement of any change in the base-line position (or change in the level of tonus maintained by the preparation), but no measure of the absolute level of tonic contraction of the preparation. The second measurement which was made was of the total amount of activity shown by the preparation during a certain period of time. The total activity depended on the amplitude and the frequency of the rhythmical contractions and was measured as the

total distance covered by the recorder pen as it moved across the chart in the given period of time.

Each worm was allowed to recover for 30 minutes after it was set up, before any recording of its activity was made. A continuous recording of the movements of the worm was then made throughout the following sequence of events:

(1) 0–20 minutes Initial period in Baldwin's medium
(2) 20–40 minutes Period in test solution
(3) 40–60 minutes Final period in Baldwin's medium

The effect of the following compounds was tested:

GABA	1×10^{-3} solution
Nicotine	1×10^{-4} solution
Serotonin	1×10^{-3} solution
D-Tubocurarine	1×10^{-3} solution
Succinyl choline	1×10^{-3} solution
Piperazine	1×10^{-3} solution

The solutions were made up in Baldwin's medium.

Results

1. The Response to Electrical Stimulation

(a) The Response to Anodal Stimulation Crossover Design Experiment

The results are given in Table I. The size of the response of each worm in each of the three solutions to a standard anodal stimulus was measured as the

TABLE I

The response to anodal stimulation

	Sequences (worms)					
Period	I	II	III	IV	V	VI
Replicate I						
1	A 15.4	B 6.0	C 16.5	A 16.2	B 5.8	C 11.8
2	B 6.6	C 10.0	A 11.5	C 10.0	A 10.0	B 10.9
3	C .6	A 10.0	B 1.0	B 0	C 10.0	A 5.7
Replicate II						
1	A 17.1	B 6.8	C 20.0	A 21.0	B 0.6	C 11.5
2	B 0	C 6.8	A 16.5	C 27.5	A 4.8	B 11.5
3	C 9.5	A 6.8	B 0	B 1.0	C 2.1	A 8.8

distance covered by the writing pen as it moved across the recorder chart. The figure obtained for each treatment was then subtracted from the figure representing the size of the response to stimulation in the initial recording period in Baldwin's medium; this allowed for variations in the size of the response in different worms. The results were then coded by adding plus 10 to each of the figures; this made subsequent calculations easier and eliminated the negative figures which were obtained when the response of a worm in a particular solution was smaller than the response of the same worm in Baldwin's medium.

The results were analyzed to test for any residual effect of the different treatments which carried over into subsequent periods. The analysis of variance

TABLE II

Analysis of variance for results given in Table I

Source	D.f.	S.S.	M.S.	*F*
Sequences (worms)	5	349.5		5.59
Periods	4	343.31		6.87*
Treatments				
Direct (unadjusted)	2	473.05		
Residual (adjusted)	2	40.30		1.63
Direct (adjusted)		495.03		19.80*
Replicates	1	8.09		
Residual (error)	21	262.58	12.50	
Total	35	1477.23		

*Indicates that the *F* value is significant at the 5% level.

is given in Table II. The *F* values are valid only when calculated for an adjusted treatment effect so that although the sum of squares for the direct treatment effect (adjusted) is not used in the analysis it is included in Table II, where it gives a measure of the significance of the treatment effects.

Although the residual effect is not statistically significant, variance associated with periods is significant, which suggests that the order of application of the three treatments determined the magnitude of their effects. Therefore, in considering the effects of A, B, and C, only the figures for period 1 are analyzed. Taking the figures for period 1 shown in Table III it is seen that

TABLE III

The effect of compounds A, B, and C on the response to anodal stimulation, taking the figure from Table I, period 1 only

Treatment	Replicate I	Replicate I	Replicate II	Replicate II	Total	Mean (coded)	Mean (uncoded)	Standard deviation
A	15.4	16.2	17.1	21.0	69.7	17.4	+ 7.4	2.48
B	6.0	.8	6.8	0.	19.2	4.8	− 5.2	?.83
C	16.5	11.8	20.0	11.5	60.1	15.0	+ 5.0	3.81

A (GABA) and C (serotonin) cause an increase in the size of the response to an anodal stimulus, whereas B (nicotine) causes a decreased response compared with the response in Baldwin's medium. Examination of the results for all three periods suggests that after treatment with B the effects of A and C are reduced, and it is possible that the residual effect of B is masked in the analysis by the absence of any residual effect of A and C.

(b) The Effect of D-Tubocurarine, Piperazine, and Succinyl Choline on the Response to Anodal Stimulation

These three substances in the concentrations tested had no effect on the response of a worm to anodal stimulation.

(c) The Effect on the Response to Cathodal Stimulation

GABA, succinyl choline, nicotine, and piperazine all inhibited relaxation in response to cathodal stimulation. The effects of serotonin and D-tubocurarine were not tested.

2. Spontaneous Rhythmical Activity

The results of the experiments in which the effect of various compounds on the frequency of spontaneous rhythmical contractions and on the tonus level of *Phocanema* was tested are shown in Tables IV and V.

TABLE IV

The effect of the various compounds on the level of tonus maintained by the preparation

Test solution	No. of worms tested	Mean change in tonus level*	Standard error of the mean
GABA	13	+ 2.46	2.24
Serotonin	7	+ 0.07	1.00
D-Tubocurarine	6	+ 0.47	1.00
Piperazine	5	+ 1.33	2.04
Succinyl choline	3	− 0.3	0.57
Nicotine	16	− 0.95	1.24

*The figure given for the mean change in tonus level represents the actual change in the length of the worm, measured in millimeters, and was calculated from the distance covered by the writing pen moving across the recorder chart.

TABLE V

The effect of various compounds on the frequency of spontaneous rhythmical contraction of the preparations

Test solution	No. of worms tested	Mean frequency in Baldwin's medium	Mean frequency in test solution	Difference between means	Standard error of the difference	t	Significance
GABA	13	12.25	6.58	5.67	3.21	1.77	Significant at P 0.1 level
Serotonin	7	20.14	12.43	7.71	3.70	2.08	Significant at P 0.1 level
D-Tubocurarine	6	9.66	17.83	8.17	6.86	1.19	None
Piperazine	5	8.25	6.25	2.0	3.51	0.57	None
Succinyl choline	3	6.0	11.0	5.0	2.33	2.15	None
Nicotine	16	9.42	8.75	0.67	2.67	0.25	None

Discussion

The effect of these compounds on the neuromuscular system of *Phocanema* is complex and does not seem related to their hyperpolarizing or depolarizing action. For example, Table VI shows that the three depolarizing agents succinyl choline, nicotine, and D-tubocurarine all have different effects on the frequency of spontaneous rhythmical contraction and on the level of tonus maintained by the worm. This does not agree with the suggestion made by Burnstock (3) that all drugs act through a membrane system and that depolarization of the membrane initiates contraction, hyperpolarization relaxation.

TABLE VI

Summary of the results of all the experiments

Test solution	Movement in response to electrical stimulation		Spontaneous rhythmical contractions (frequency)	Tonic contraction (tonus level)
	Anodal (contraction)	Cathodal (relaxation)		
GABA	No effect	Inhibition	Decrease	Decrease
Serotonin	No effect	?	Decrease	No change
Succinyl choline	No effect	Inhibition	[Increase]	No change
Nicotine	Inhibition in some cases	Inhibition	No change	Increase
Piperazine	No effect	Inhibition	No change	Decrease
D-Tubocurarine	No effect	?	[Increase]	Slight decrease

[] indicates that the figures were not statistically significant, but showed a trend towards the result given.
? indicates that there are no figures for this result.

Although it is not possible to discuss these results in relation to the observations which were made of the response of a nematode worm to external depolarizing and hyperpolarizing stimuli (1), there are some interesting conclusions which can be drawn from the summary in Table VI.

The frequency of rhythmical contraction may increase (with succinyl choline) or decrease (with GABA or serotonin) with no corresponding change in the level of tonus maintained by the worm. Conversely, the tonus level may increase (in nicotine) or decrease (in piperazine) without any corresponding change in the frequency of rhythmical contraction. These observations suggest that rhythmical activity and tonic contraction are the products of two independent systems and that they can be controlled separately.

In the discussion of some earlier experiments with *Ascaris* some attempt was made to relate the response of the worm to electrical stimulation to the way in which its rhythmical movements developed (1). The results of the present series of experiments, however, show that one cannot relate changes in rhythmical activity to any changes in the ability to respond to electrical stimulation. Thus, in GABA or serotonin there is a decrease in the frequency of rhythmical contraction, and in succinyl choline there is an increase in frequency, associated in each case with the inhibition of the ability of the worm to respond to cathodal stimulation. In all cases there is no corresponding change in the ability to respond to anodal stimulation. The response to cathodal stimulation may be blocked with no change in the rhythmical activity of the preparation (for example, in nicotine or piperazine). The rhythmical movements of a preparation may be affected without any associated change in the response to an anodal stimulus. These results suggest that the rhythmical activity of *Phocanema* is not controlled by the same mechanism which is responsible for the response to electrical stimulation; it seems possible that the rhythmical activity is myogenic rather than neurogenic, although the rhythmical behavior may be modified by the action of motor nerves (which could be excitatory or inhibitory). This is in agreement with the conclusions reached in the discussion of the experiments on the effect of cold on the response of *Phocanema* to electrical stimulation (2).

The level of tonus which is maintained by the preparation can also be changed independently of the response to electrical stimulation. Thus, in GABA or piperazine there is a decrease in the tonus level of the worm and in nicotine there is an increase which is associated in both cases with the loss of the ability to respond to cathodal stimulation. There is no associated change in the response to anodal stimulation. Thus neither the rhythmical nor the tonic contractions of *Phocanema* appear to be associated with the response to cathodal stimulation.

The response to cathodal stimulation is inhibited by GABA, succinyl choline, nicotine, and piperazine. It is interesting to notice that when relaxation is blocked, the preparation does not respond to cathodal stimulation by contracting, although one would have expected that the other electrode (the anode) would have acted as the stimulating electrode under these circumstances. The reason for this is not understood.

The results of these experiments suggest that in *Phocanema* rhythmical movements and the maintenance of a certain degree of tonic contraction are produced by two different, but not necessarily independent effector systems. There is nothing in the results which indicates whether these two effector systems are present in each muscle cell or whether the fast and slow contractions are produced by different cells. It is not possible to relate the response to either anodal or cathodal stimulation to the development of either fast rhythmical or slow tonic contractions, although it seems most improbable that the response to stimulation would be mediated through a third effector system.

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THE EFFECTS OF ULTRAVIOLET RADIATION ON THE INFECTIVE STAGES OF TRICHOSTRONGYLUS RETORTAEFORMIS ZEDER (NEMATODA)[1]

S. P. GUPTA[2]

Abstract

The effects of three ranges of ultraviolet radiation on larvae of *Trichostrongylus retortaeformis* have been studied. It is shown that the far ultraviolet has a quickly lethal effect on the larvae, the middle ultraviolet has a much lessened effect, and the near ultraviolet has practically no effect. Larvae not killed by the ultraviolet radiation develop normally in rabbits.

Introduction

During their free life the larvae of parasitic nematodes are exposed to a variety of external influences, such as the temperature and relative humidity of their microenvironment, oxygen tension and other chemical influences, and the effect of that portion of the electromagnetic spectrum which reaches the surface of the ground from the sun. This portion is conventionally divided into three sections:

(*a*) the visible section which ranges from the red end (at about 7880 Å) to the violet end (at about 3800 Å);

(*b*) the infrared (i.r.) portion (above 7880 Å); and

(*c*) the ultraviolet (u.v.) portion (below 3800 Å).

It is known that the ultraviolet portion has definite effects on various organisms and the purpose of the experiments described in this article was a preliminary examination of these on the free-living larvae of *Trichostrongylus retortaeformis* of the rabbit.

The ultraviolet range of the spectrum is conventionally divided into three portions:

near u.v.	from 3800 to 3200 Å,
middle u.v.	from 3200 to 2800 Å,
far u.v.	from 2800 Å.

This division has been made on practical grounds. The near u.v. has the power of exciting certain substances to fluoresce and is often referred to as the *photochemical* region. The middle u.v. produces reddening of the human skin and is often called the *erythemal* range. The far u.v. is capable of killing bacteria and other organisms and is referred to as the *germicidal* range. Only the near and the middle portions normally reach the earth from the sun, although its energy output comprises the entire spectrum. However, even within these limits, there is no constancy in the amount of u.v. reaching the earth and so, to obtain consistent results, advantage was taken of the ability of the mercury arc to cover the near, middle, and far portions.

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The mercury arc, unlike the sun, does not produce a continuous band spectrum but a line spectrum in which there are rather sharply defined lines, separated by intervals in which no energy is produced. The extent to which the bands are separated depends to some extent on the pressure of the gas within the discharge tube. In some cases, these energy bands can be used to excite broad continuous bands over limited portions of the spectrum by the use of phosphorescent and fluorescent substances on the inside of the discharge tube.

While the energy released in the u.v. range from the mercury arc is high, much of it can be filtered out by the translucent material of which the tube is made. Some substances, like quartz, permit almost all to pass into the air, while glass has a variable effect, ranging from almost all to none, depending on its chemical composition.

Accordingly, to find the qualitative effect of various regions of the u.v. portion of the spectrum, it is necessary to employ mercury arc lamps, together with suitable filters to restrict portions of the range.

The lamp used in these experiments, a UA-2,[3] is an excellent source of all three divisions of ultraviolet. It is a limited pressure lamp enclosed in a quartz envelope which emits bands of energy from 6234 Å to 2200 Å: the bands in the ultraviolet which pass the quartz envelope are depicted at the top of Fig. 1.

By the use of this lamp and suitable Corning[4] glass filters, it is possible to restrict the range of its u.v. radiation. In this way larvae can be exposed to all three ranges, to the middle and longer ranges, to the longer range alone, or they can be shielded completely.

The filters employed, their Corning numbers, and their transmission characteristics are shown in Fig. 1.

All filters have a more or less definite cutoff in the lower range of the spectrum. Filter A transmits 70% of the far u.v., while filter B transmits only 40%; B, however, unlike A, transmits no infrared. Filter C transmits about 60% of the middle u.v. range, while filter D transmits very little. Filter E transmits about 90% of the near u.v. but no i.r. Filter F has about the same range in the u.v. and visual portion of the spectrum as has sunlight but only transmits near i.r.

Filters F, G, and H transmit some near u.v.; filter F transmits no i.r.; filter G some near i.r.; and filter H no green-yellow rays. Filters K, L, M, and N transmit practically no u.v., while filter N transmits no i.r. as well.

All mercury arc lamps and some of the filters pass visible light. Accordingly, a series of tests was carried out with a variety of colored gelatine filters which individually and collectively covered all portions of the visible portion of the spectrum from 3200 Å into the infrared. The tests were carried out under the same conditions as those involving ultraviolet radiation. In no case was any appreciable effect seen and it is concluded that the visual portion of the spectrum could be ignored in these experiments. In addition, moreover, some of the filters excluded visible light but passed the same range of u.v. as others which also passed visible light. No significant differences were noted between them.

[3]Made by the General Electric Co., U.S.A.
[4]Made by the Corning Glass Co., Corning, N.Y.

Previous work (1) has shown that the effect of heat on the length of life of the infective larvae of this nematode increases considerably at temperatures over 25° C. Accordingly, these experiments with the UA-2 lamp were conducted at this temperature. This was done by suspending the lamp 4½ inches above the culture and placing the larvae and the filters under test in the path of a continuously operating electric fan. A thermometer beside the culture dishes registered a temperature of between 24° C and 25° C.

Although the published characteristics of the lamp used showed no i.r. radiation, a series of tests with an i.r. lamp was carried out. This lamp is a filament one, operating at about 2500° K, generating no u.v. but considerable short-wave i.r. radiation; visual radiation was largely prevented by a red glass filter.

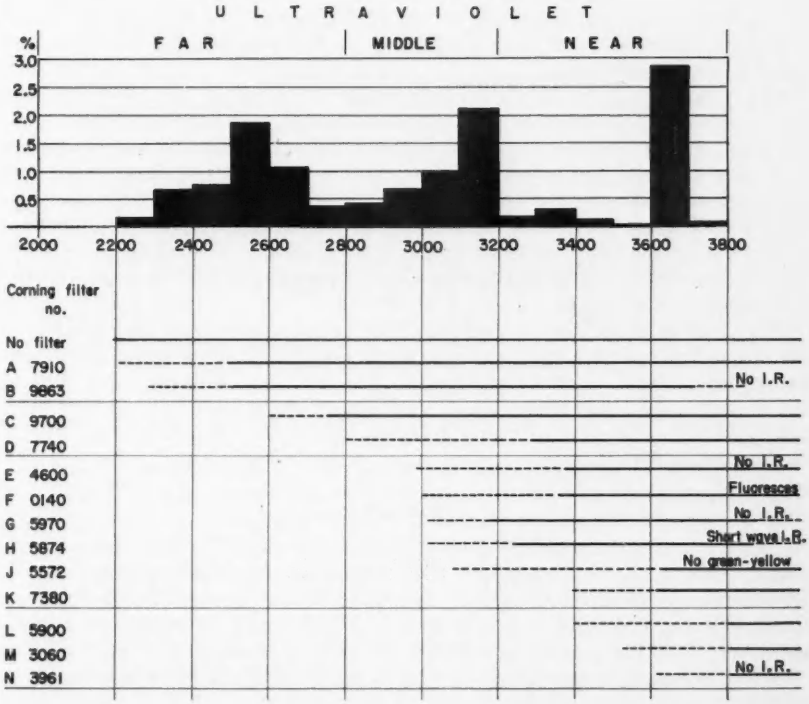

FIG. 1. The top portion of the diagram represents the energy output in the u.v. of the UA-2 lamp employed in these experiments.

The lower section represents the transmitting portions of the Corning filters used: the unbroken line shows the important bands transmitted, while the broken part of the line shows a negligible extension. Most filters extended into the visible range but filters B, G, and H passed virtually no visible rays; these filters together with filter N were opaque or almost so to the i.r.; filter E, which also is opaque to the i.r., passes visible light.

As in the u.v. experiments, fans were used to dissipate the heat generated by the lamp and radiated outwards. Thermometers placed beside the culture dishes did not exceed 25° C, a temperature which is within the range tolerated by these larvae. Several experiments showed that, when petri plates containing the larvae in 5 ml of distilled water were suspended 16 inches from the lamp and 12 inches above the table, the death rate among the larvae was negligible and corresponded closely with controls held at room temperature.

In addition certain u.v.-transmitting filters used were opaque to the i.r. radiation: no significant difference was observed between them and those passing i.r.

A small amount of ozone is produced by the UA-2 lamp, although its maximum production is probably due to shorter wave lengths than those which pass its quartz envelope. However, the amount produced was detectable by its smell, but the fan, playing continually over the cultures to replace heated air, probably rendered negligible the effect of the ozone on the larvae.

In each experiment 100 healthy larvae were placed in about 5 ml of distilled water in 2-in. petri plates.

The larvae employed in these experiments were healthy infective larvae of *Trichostrongylus retortaeformis*. Unlike those which were used in determining the effect of temperature, they were raised in faecal cultures, mixed with sand and charcoal, and incubated at about 25° C. After 4 days' incubation, the larvae were recovered by the Baermann technique. About 100 larvae were held in 3-in. petri plates and covered either with a quartz plate or an appropriate filter. They were exposed to the specific radiation until all were dead; during this time distilled water was added from time to time to make up the loss due to evaporation.

In an earlier paper (1) it was shown that larvae which had been raised from the egg to the infective stage on agar cultures of *Escherichia coli* at 25° C, and subsequently held at 25° C, all died within 143 days, whereas those held at 30° C died in half that time. There were practically no deaths in either range before 44 and 8 days, respectively, but 80% had died in 101 days at 25° C and in 50 days at 30° C.

Results

Table I shows the average approximate times taken for 100 larvae to die and is based on several experiments in each case. In all experiments the extremes were close to the average. The results are, of course, qualitative as, under the conditions of the experiment, it is impossible to make accurate estimates as to the amount of energy applied to the larvae.

The UA-2 lamp consumes 3.1 amperes at 110 volts. It dissipates considerable heat but it also radiates

7.56 watts in the far, or germicidal, range of u.v.;
9.81 watts in the middle, or erythemal, range of u.v.;
8.79 watts in the near, or photochemical, range of u.v.; and
17.90 watts in the visible range.

It has been shown that visible light has no effect on the longevity of the larvae and we can neglect it for the present. Heat is dissipated by an electric fan.

It should be noted that approximately equal quantities of energy are emitted in the u.v. range. Accordingly, the proportion of energy available to the first group of filters is 3, to the second 2, to the first 1. As these experiments were designed on the quality of this energy rather than the quantity, it would be expected that death rates would be approximately in the reciprocal proportions to the energy applied, that is, those exposed to the first group would be killed in one-third of the time, those exposed to the second group in half the time of those in the third group, while those in the fourth group would have a normal life expectancy, provided the energy absorbed by the larvae did not alter according to the wave length but only according to the wattage. This was not the case.

TABLE I

Average time required to kill larvae exposed to radiation with a UA-2 lamp

u.v. range	Filter	Time
All available u.v. rays	—	0.66 hours
	A	2.5 hours
	B	4.25 hours
All available over 2700 Å	C	118 hours
	D	360 hours
All u.v. rays over 3100 Å	E	19 days
	F	17.6 days
	G	37 days
	H	42 days
	J	24 days
	K	40 days
No rays under 3700 Å	L	38 days
	M	30+ days
	N	30+ days

Several of the filters used were in the nature of controls. However, filters A, C, and E can be regarded as permitting, respectively, the passage of all three ranges of u.v., two longer ranges, and the near u.v., while filter N permits no u.v. to pass. The times for the death of all larvae were, respectively, 2.5 hours, 118 hours, 19 days, and over 30 days.

Filter A requires about half the time required by filter B to kill all the larvae. Filter A transmits nearly twice the amount of far u.v. as filter B; however, both filters pass middle and near bands as well as the far u.v. Filters C and D pass middle and far u.v., and both take much longer to kill the larvae than filters A and B. Filter C is much more effective than filter D in the middle range and is about three times as lethal. Filter E passes a considerable quantity of photochemical rays, while filter F passes much the same u.v. as is found in sunlight. Both are slightly more lethal than the remaining filters which are nearly or quite opaque to the u.v.

As all measurements were made at the same distance from the UA-2 lamp and at the same temperature, these results suggest that the far u.v. is much

more lethal than the middle or near ranges, but that the middle range also has some lethal effect, while the near range has little or no effect.

It is apparent that sufficient exposure to the middle and especially the far u.v. range can cause death outside of the host. However, it might also prevent the larvae from developing once they entered the host, if exposure had been long enough. To test this, larvae were exposed to a low-pressure fluorescent lamp with a quartz envelope which radiated 98% of its u.v. energy at 2537 Å. Batches of a thousand larvae, cultured as before, were exposed for 2, 4, 6, 8, 10, 12, 14, and 16 minutes. Each batch was fed to a rabbit and the animal was killed 2 to 3 weeks later. Two rabbits were used for each batch of larvae. No adults developed from larvae radiated for 14 and 16 minutes, 1% from those radiated for 12 minutes, 2% from those radiated for 10 and 8 minutes, 2.5% from those radiated for 6 minutes, 3% from those radiated for 4 minutes, and 4% from those radiated for 2 minutes.

A careful morphological examination of adult worms, developed from larvae radiated for 6, 10, and 12 minutes, showed no morphological changes.

While the decreasing percentage of recovery may have some significance, the numbers used were too small to draw any conclusions beyond the fact that the germicidal range can prevent development of larvae to adult life, even when it does not cause death. However, adults raised from larvae which survived radiation for 12 minutes were found to produce eggs. From these an F_1 generation was cultured which successfully infected a rabbit, again producing normal adults.

Reference

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A KEY TO THE SPECIES OF THE NEMATODE GENUS APHELENCHOIDES FISCHER, 1894[1]

K. C. SANWAL

Abstract

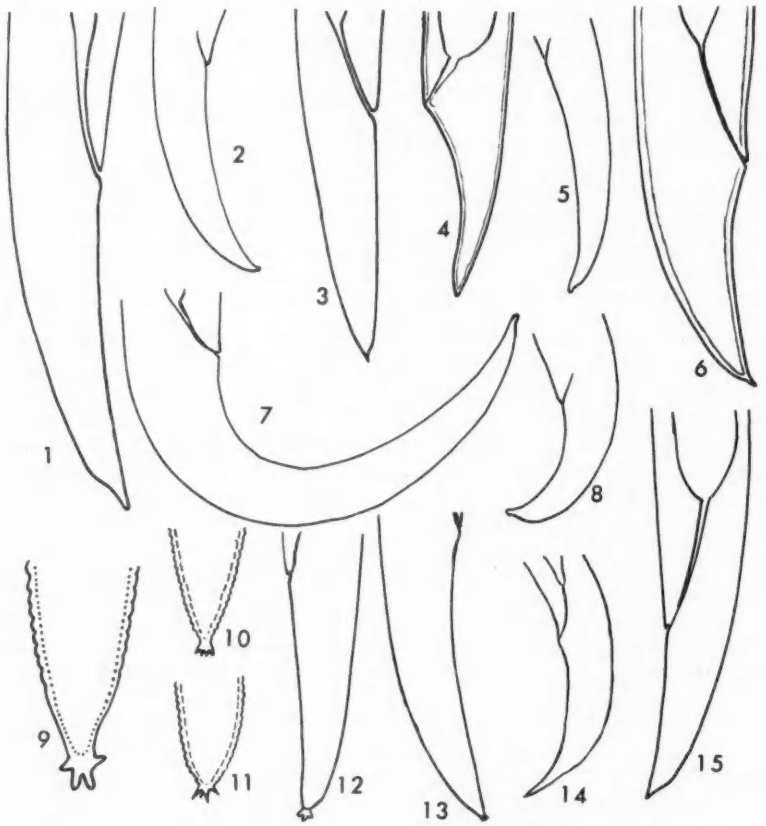
A key for the identification of 35 species of the nematode genus *Aphelenchoides* Fischer, 1894 is given. The key is based on the characters of the females. The chief characters used in the key are as follows: shape of female tail, structure of stylet, presence or absence of postvulvar uterine sac, position of excretory pore and nerve ring, and number of lateral incisures. The descriptive terms used in the key are illustrated by line drawings. A separate key is provided for species where only males are known.

The nematode genus *Aphelenchoides* Fischer (12) is represented by a large number of species, many of which are of world-wide distribution and are found free-living in soil, parasitic in plants, and associated with insects. For a number of years this genus included a heterogenous congregation of species which have since been found to belong to more than one genus of the Aphelenchoidea. This was mainly due to a lack of closer understanding of the differentiating characters of some genera of the Aphelenchoidea which, at first, showed considerable overlapping. J. B. Goodey (17) has recently published a revised classification of the Aphelenchoidea. Although I do not agree completely with the changes and additions effected by Goodey, his paper has clarified some aspects of the taxonomy of this group.

In the present paper a key to the species of *Aphelenchoides* Fischer, 1894 is presented for the identification of different species of the genus. The key is based on characters of females. For the two species of *Aphelenchoides* of which only males have been found, a separate key is given.

J. B. Goodey (17) lists 34 species of the genus *Aphelenchoides*. He does not include the following species, either in his list of valid species and synonyms, or in the list of *species inquirendae*: *A. clarolineatus* Baranovskaya, 1958 (2), *A. martinii* Ruehm, 1955 (28), *A. teres* (Schneider, 1927) Filipjev, 1934 (30, 10), *A. zeravschanicus* Tulaganov, 1949 (43), and *A. spinocaudatus* Skarbilovich, 1958 (32). Of these I regard *clarolineatus* and *martinii* as valid species and these are included in the key; *teres* and *zeravschanicus* are regarded as *species inquirendae*; and *spinocaudatus* is regarded as *nomen nudum*. *A. caprifici* (Gasparrini, 1864) Filipjev, 1934 (16, 10) is not included in the key as I do not agree with J. B. Goodey in placing this species in the genus *Aphelenchoides*. Cobb (7) definitely mentioned that the spear is cleft at the base. This phenomenon of a posteriorly split spear, being by no means uncommon in the nematode organization, seems to be a valid character to delimit the subgenus *Schistonchus* Cobb, 1927 (7) from other genera of the Aphelenchoidea. Skrjabin *et al.* (33) raised the subgenus *Schistonchus* to generic rank, but no mention of this publication or the views expressed therein has been made by J. B. Goodey. I am in

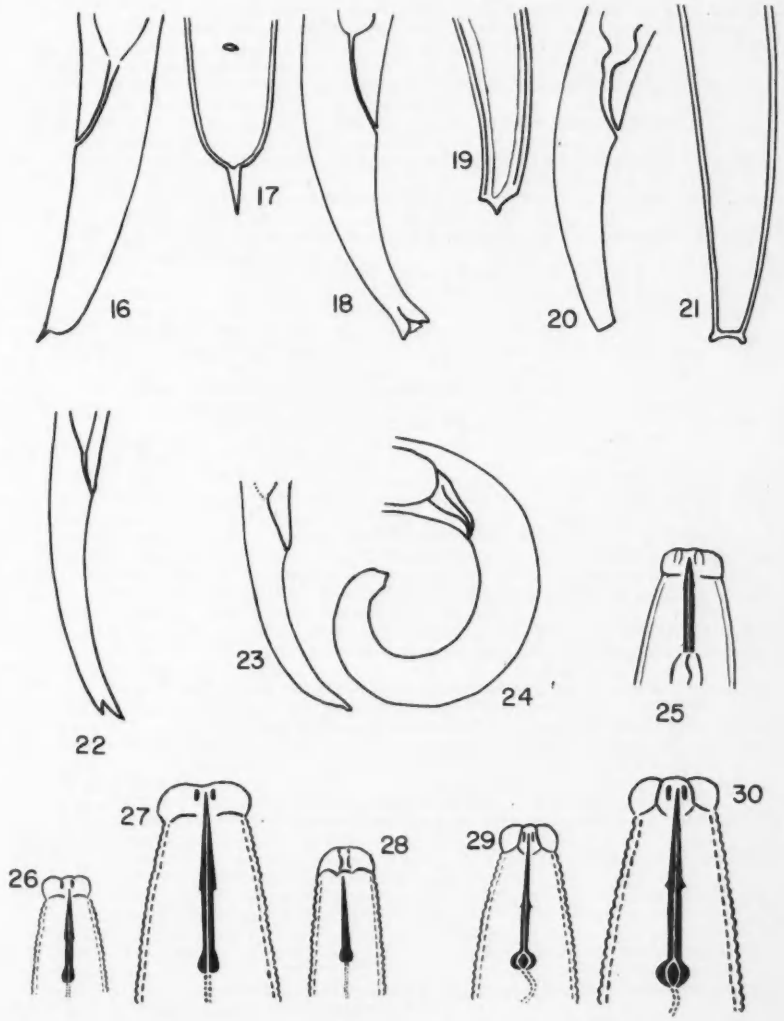
[1]Manuscript received December 21, 1960.
Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.



FIGS. 1–15. Various shapes of female tails.

complete agreement with Skrjabin *et al.* (33) in as far as the validity of the genus *Schistonchus* is concerned. The composition of the genus, however, may be debatable.

While using the following key it should be borne in mind that a key is only an aid to identification. The final identification reached through the key should invariably be confirmed through comparison with the original description and illustration of a species. Another point to remember is that the characters used in the key are not necessarily diagnostic characters but are only differentiating characters. The illustrations given with the key are freehand drawings of tail ends of different species and different types of stylets. They are chiefly intended to interpret the author's conception of the descriptive terms used in the key, and may not be the exact drawings of the different species, although they have been adapted from the original drawings. A reference to the original



FIGS. 16–24. Various shapes of female tails. Figs. 25–30. Types of stylet.

description and illustration for the final confirmation of the identification reached through the key is, therefore, essential.

1. Female tail conoid, distinctly tapering to a sharp or rounded point, with or without mucronate structures at tail tip (Figs. 1–15)..... 8
2. Female tail not tapering to a sharp or rounded point as in 1, but narrowing to an obtusely rounded or truncate terminus with or without mucronate or other kinds of structures at tail tip (Figs. 16–21)..... 5

3. Female tail with a bifurcated tip (Fig. 22) 6
4. Female tail elongate cylindrical, cylindroconical, finger-shaped (Figs. 23, 24) 7
5 (*a*). Tail tip with three leaflike expansions (as in Fig. 18) ..... *sphaerocephalus* Goodey, 1953 (20)
(*b*). Tail terminus bluntly rounded with a ventrally situated mucron (as in Fig. 16) .. *subtenuis* (Cobb, 1926) Steiner & Buhrer, 1932 (6, 40)
(*c*). Tail short, bluntly rounded with a terminally situated filiform mucron (as in Fig. 17) ..... *martinii* Ruehm, 1955 (28)
(*d*). Tail tip obtusely rounded to truncate, without any mucronate structure (as in Fig. 20) ..... *limberi* Steiner, 1936 (37)
(*e*). Tail truncate with two small hornlike projections (as in Fig. 21) ..... *kungradensis* Karimova, 1957 (23)
(*f*). Tail truncate with a triangular chisel-shaped tip (as in Fig. 19) ..... *clarolineatus* Baranovskaya, 1958 (2)
6 (*a*). Female length, 0.38–0.47; *a*, 31.3–31.7; *b*, 6.8–8.4; *c*, 9.4–12.6 ..... *bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941 (22, 12)
(*b*). Female length, .900–1.3; *a*, 42.5–46.1; *b*, 11.2–13.4; *c*, 14–16.7 ..... *hainanensis* (Rahm, 1938) Goodey, T., 1951 (26, 19)
7 (*a*). Postvulvar uterine sac absent, female tail elongate cylindrical, finger-shaped, 7.5 anal diameters long (as in Fig. 24) ... *abyssinicus* (Filipjev, 1931) Filipjev, 1934 (9, 10)
(*b*). Postvulvar uterine sac present, female tail shorter than in *abyssinicus*, cylindrical, finger-shaped (as in Fig. 23) ..... *dactylocercus* Hooper, 1958 (21)
8. Female tail with or without a single, simple mucor at tail tip 12
Female tail always with more than one mucronate structure or star-shaped structure at tail tip (as in Figs. 9–13) 9
9. Female tail with four mucronate structures at tip (as in Figs. 10, 11, 13) 10
Female tail with a distinct star-shaped structure at tip (as in Figs. 9,12) 11
10. Excretory pore anterior to nerve ring, postvulvar uterine sac short, narrow, less than 4 times body width, four mucronate structures at tail tip (sometimes divergent and may give the appearance of a star (as in Figs. 10, 11) ..... *besseyi* Christie, 1942 (5)
Excretory pore posterior to nerve ring, postvulvar uterine sac five or more body widths long, four mucronate structures at tail tip (as in Fig. 13) ..... *ritzema-bosi* (Schwartz, 1911) Steiner & Buhrer, 1932 (31, 39)
11 (*a*). Lateral fields with two incisures (tail shape as in Fig. 12) .. *asterocaudatus* Das, 1960 (8)
(*b*). Lateral fields with three incisures (tail shape as in Fig. 9) ..... *nonveilleri* Andrassy, 1959 (1)
(*c*). Lateral fields with four incisures .. *coffeae* (Zimmerman, 1898) Filipjev, 1934 (44, 10)
12 (*a*). Stylet simple, without any basal thickenings, swellings or knobs (Fig. 25) 13
(*b*). Stylet with distinct and clearly defined knobs (Figs. 29, 30) 22
(*c*). Stylet without distinctly formed knobs as in 12*b*, but with basal thickenings, basal swellings or faint knobs (Figs. 26–28) 14
13. Postvulvar uterine sac absent, tail short, 3–3½ times anal diameters long, slightly swollen at the end ..... *chamelocephalus* (Steiner, 1926) Filipjev, 1934 (35, 10)
Postvulvar uterine sac present, tail more than five anal diameters long (as in Fig. 7) .. *longiurus* Das, 1960 (8)
14. Postvulvar uterine sac present 15
Postvulvar uterine sac absent, anterior end of body cylindrical, not attenuated, head not set off ..... *goeldii* (Steiner, 1914) Filipjev, 1934 (34, 10)
15. Postvulvar uterine sac well developed, more than 1½ times body diameter 16
Postvulvar uterine sac rudimentary, not more than 1½ times body width (male tail with a sharp setoff terminal spine ..... *spinosus* Paesler, 1957 (25)
16. Lateral fields present 17
Lateral fields absent, excretory pore behind level of nerve ring ..... *helophilus* (de Man, 1880) Goodey, T., 1933 (24, 18)
17. Lateral fields with four incisures at middle of body 18
Lateral fields with three incisures at middle of body 21
18. Nerve ring almost immediately behind median esophageal bulb 19
Nerve ring about one body width behind median esophageal bulb 20
19 (*a*). Proportion between length and breadth of egg 2.6:1 ... *saprophilus* Franklin, 1957 (15)
(*b*). Proportion between length and breadth of egg 4:1 ..... *parietinus* (Bastian, 1865) Steiner, 1932 (3, 36)
20. Stylet thickenings slightly heavier than in *parietinus*, worms lie in the shape of a bow when relaxed by heat ..... *cyrtus* Paesler, 1957 (25)
21. Eggs 46 by 19 μ; *c*, 14(11–17) ..... *composticola* Franklin, 1957 (15)
Eggs 38 by 15 μ; *c*, 18(13.8–24.7); tail rather wide as compared to *composticola* ..... *sacchari* Hooper, 1958 (21)

22. Postvulvar uterine sac present 23
Postvulvar uterine sac absent (tail shape as in Fig. 15) ..... *tagetae* Steiner, 1941 (38)
23. Lip region distinctly demarcated from body 24
Lip region not demarcated from body, almost continuous with neck contour, lateral fields with two incisures ..... *fragariae* (Ritzema Bos, 1890) Christie, 1932 (27, 4)
24. Stylet not more than 12 μ long 25
Stylet more than 12 μ long 27
25. Excretory pore behind the level of median bulb 26
Excretory pore at level of median bulb, stylet 8 μ long with thin, short tip, tail wedge-shaped (as in Fig. 14), associates of insects ..... *sinodendroni* Ruehm, 1957 (29)
26. Stylet 6.2 μ long, tail ending in a mucron formed by a sudden constriction of terminus (as in Fig. 1) ..... *pusillus* (Thorne, 1929) Filipjev, 1934 (42, 10)
Stylet 12 μ long, tail tip with a single, simple fine mucron (as in Fig. 6) . *singhi* Das, 1960 (8)
27. Tail gradually narrows to tip 28
Tail narrows sharply on the ventral side after anus (as in Fig. 4), stylet 15 μ long ... . *scalacaudatus* Sudakova, 1958 (41)
28. Stylet 14 μ long, tail without a mucron at tip ..... *kuehnii* Fischer, 1894 (12)
Stylet 17 μ long with a simple mucron at tail tip (as in Fig. 3) .... *blastophthorus* Franklin, 1952 (13)

Key to Species Represented by Males Only

1. Median esophageal bulb oval, tail short and bulky, about two anal diameters long, without a mucron ..... *brevicaudatus* Das, 1960 (8)
Median esophageal bulb triangular in shape, tail not short and bulky as in *brevicaudatus*, about three anal diameters long, with a distinct, well-developed mucron .. .. *brevionchus* Das, 1960 (8)

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DISTOMIASIS IN TOKELAU ISLANDERS[1]

MARSHALL LAIRD

Abstract

Embryonated eggs of a trematode, presumably a heterophyid acquired by eating raw or inadequately cooked fish, were found in faecal concentrates from 13 (4%) of 324 Tokelauans. Positive samples originated from all three atolls of the New Zealand dependency, Atafu (08° 33′ 30″ S., 172° 30′ W.), Nukunono (09° 10′ S., 171° 50′ W.), and Fakaofo (09° 23′ S., 171° 14′ W.). Eleven of those concerned had never travelled beyond the Tokelau Group, making this the first center of autochthonous distomiasis known in the South Pacific.

Introduction

In September and October, 1958, and again in April, 1960, vector control experiments were undertaken at Atafu (08° 33′ 30″ S., 172° 30′ W.), Nukunono (09° 10′ S., 171° 50′ W.), and Fakaofo (09° 23′ S., 171° 14′ W.). These three Polynesian atolls are New Zealand dependencies. Together with a fourth, the American island of Olosenga (11° 05′ S., 170° 55′ W.), they form the Tokelau or Union Group. The project took place under the auspices of the World Health Organization and the New Zealand Government, and called for initial estimates of microfilaraemia due to *Wuchereria bancrofti* at each island. Little being known of the intestinal parasitology of the inhabitants, the opportunity was taken to obtain faecal samples too. Full accounts of the experiments and parasite survey are being published elsewhere, this paper only dealing with the discovery of autochthonous fluke infections, hitherto unknown in the South Pacific.

Discounting the numerous cercariae responsible for schistosome dermatitis, some 60 species of trematodes have been identified from man. At least 37 (70% of them mere accidental parasites of the intestine) affect peoples of the eastern borders of the Oriental and Palearctic Regions, who are especially liable to infection through their common habit of eating raw or inadequately cooked fish (9). This practice extends throughout the Pacific, as do melanid snails, fish, and piscivorous birds, which are successive hosts for flukes capable of infecting man.

However, most of the handful of records of distomiasis from the Central and South Pacific concern persisting clonorchiasis or paragonimiasis in Oriental immigrants; or freak infections—clonorchiasis in Hawaii (8), opisthorchiasis in the Society Islands (5)—attributable to the eating of imported fish bearing encysted metacercariae. Locally acquired distomiasis has been reported only from Hawaii, where *Fasciola gigantica* Cobbold has been blamed for 19 cases of human fascioliasis (1), and two infections with *Stellantchasmus falcatus* Onji and Nishio have been described (2, 4). At least three heterophyids additional to the latter one pose health hazards in Hawaii (7), but it should be

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TABLE I

Heterophyid infection of Tokelau Islanders by age groups, September/October, 1958

Age group (years)	Locality							
	Fakaofo		Nukunono		Atafu		All three atolls	
	Examined	Positive	Examined	Positive	Examined	Positive	Examined	Positive
0– 9	22	1	7	0	2	0	31	1
10–19	24	0	21	2	23	2	68	4
20–29	29	2	33	3	9	0	71	5
30–39	20	2	16	1	27	0	63	3
40–49	11	0	14	0	18	0	43	0
50–59	8	0	6	0	7	0	21	0
60+	6	0	3	0	18	0	27	0
Total	120	5	100	6	104	2	324	13

understood that this seeming prodigality may merely reflect the fact that relevant research has been more actively prosecuted there than elsewhere in Oceania.

Materials, Methods, and Results

Pea-sized aliquots of stools obtained from 324 islanders in 1958 were preserved in MIFG solution and later subjected to formalin–ether sedimentation as recently detailed (6). On examination by members of this Institute's 1959 refresher course for laboratory technicians, 13 of the samples yielded embryonated trematode eggs (Table I).

The eggs were oval, lacking knobs, spines, or other ornamentation. There was an operculum at the narrower end, often difficult to make out because of its inconspicuous shoulders. Fifty examples measured from 26.1 to 30.8 μ by from 19.0 to 24.0 μ (av., 28.3 by 21.5 μ). The shell ranged from 1.2 to 2.0 μ in thickness, and its dark yellowish-brown color may have been intensified by the iodine of the preservative.

Those parasitized (Table I) were from 5 to 34 years of age, 11 (85%) of them being females. Two had visited Samoa, one had not been away from the home island (Fakaofo), and the remainder, while never having travelled beyond the New Zealand dependency, had visited at least one of its other atolls. The infection, which has an over-all incidence of 4%, is thus autochthonous as regards these islands in general and Fakaofo in particular.

Shortage of time during the 1960 visit limited further investigations to (*a*) the obtaining of purged stools (which proved uniformly negative) from 10 of those parasitized a year and a half previously and (*b*) the shooting of a number of adult terns for examination for mature trematodes—seven *Anous minutus minutus* Boie, four *A. stolidus pileatus* (Scopoli), three *Gygis alba candida* (Gmelin), and one *Sterna sumatrana sumatrana* Raffles. The tract from stomach to cloaca was removed, slit lengthwise, and preserved in 5% sea water – formalin. A subsequent search failed to reveal any helminths other than a few cestodes in one of the *A. s. pileatus*.

Discussion

Knowledge of the parasite thus remains limited to its egg, the morphology and minuteness of which suggest that it is referable to the Heterophyidae. The short-lived nature of the complaint and its low incidence in three congested populations each inhabiting a single small islet of the atoll concerned would agree with a diagnosis of accidental infection by a member of this family. Although the egg cannot be assigned to any fluke previously recorded from man, and is certainly not referable to *Stellantchasmus falcatus* as described by the Hawaiian investigators (2, 4), it strongly resembles that of *Apophallus venustus* (Ransom). The latter species is listed from man in Canada, and its broadly oval egg, in which the operculum is sometimes seen only with difficulty, measures from 26 to 32 μ by from 18 to 22 μ (3).

Only one melanid snail and suspect first intermediate host for heterophyids, the littoral to fresh-water *Melanoides tuberculata* (Müller), is known from the Tokelau Islands. Young mullets, *Mugil* spp. (good second intermediate hosts) and *Neomyxus chaptalii* (Eydoux and Souleyet), are prominent among the

fishes sharing the environment of this mollusc. Tokelauans frequently chew upon mullet fresh from the lagoon, and the fact that between five and six times as many females as males harbored flukes could well be explained by the women's virtual monopoly on inshore fishing. Piscivorous birds feeding in this same area, and regarded as the group most likely to furnish the normal definitive host or hosts, are the four terns already mentioned and a resident shore bird, the reef heron, *Egretta sacra sacra* (Gmelin). It is not without interest to recollect that herons are prominent as definitive hosts for *Apophallus* (3) among other heterophyids.

Further visits to the Tokelau Islands are not presently contemplated, but similar epidemiological circumstances exist at other atolls and elsewhere in Oceania. These data are thus submitted for the purpose of alerting local medical authorities to the possibility of encountering accidental distomiasis, and to sketch a background for the investigation of life histories.

Acknowledgments

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THE IN VITRO HATCHING OF ASCARIS LUMBRICOIDES EGGS[1]

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Abstract

The stimulus leading to in vitro hatching of *Ascaris lumbricoides* eggs consisted of four components as follows: (1) a temperature similar to that of homoiothermic animals, (2) pCO_2 approximating 5 vol.%, (3) pH near 7.0, (4) nonspecific reducing conditions such as are established by cysteine, glutathione, sodium hydrosulphite, sodium bisulphite, or sulphur dioxide. Application of this stimulus resulted in 80–95% hatching in 3 hours, the observed morphological changes corresponding to those occurring during intestinal hatching. The stimulus was not effective when applied to preinfective stages of embryonic development, or to infective eggs which were embryonated in 2% potassium dichromate solution. Maximal hatching was obtained when the stimulus was applied continuously. One of the first results of stimulation may be increased permeability of the vitelline membrane sufficient to permit the outward passage of enzymes such as chitinase which digest the chitinous shell. There is no reason to believe that the in vitro stimulus differs significantly from the natural stimulus, which is common to many homoiothermic animals and may account in part for the low degree of host-specificity in *Ascaris* infections.

Introduction

Rogers (13, 14) continued an investigation into the mechanism of hatching of infective *Ascaris lumbricoides* eggs begun in this laboratory by B. I. Passey (unpublished results). Normally, these eggs remain dormant (10, 11) until they enter the host intestine, where they hatch in about 2 hours. Apparently the intestinal environment quickly enables the embryo to escape from the egg despite the presence of an unusually tough and durable shell (4). In vitro, hatching was shown to result from the liberation of chitinase and possibly of other enzymes within the egg, the stimulus for this release being provided by external conditions which included a low oxidation–reduction potential and an abundant supply of free carbon dioxide. Under these conditions, a hole was digested in the chitinous shell through which the vitelline membrane protruded as a bubble, the head region of the infective embryo usually being inserted in this bubble and ultimately breaking through it. Maximal hatching occurred in 2 to 3 hours. Because identical morphological changes in the shell were observed in hatching eggs recovered from the intestine of laboratory animals, in which reducing conditions and a relatively high partial pressure of carbon dioxide were to be expected, the in vitro stimulus was believed to be physiological at least in the sense that it was compatible with naturally occurring conditions. Moreover, unpublished experiments had indicated that in fasting mice egg-hatching was greatly reduced, a result consistent with the idea that the intestine under these conditions possessed a relatively high oxidation–reduction potential and low partial pressure of carbon dioxide.

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The present report is concerned primarily with a more detailed investigation of the stimulus, and its effects on eggs subjected to different environmental conditions.

Methods

Preparation of Eggs for Hatching

Eggs were collected from the uteri of *Ascaris* females, decoated, and embryonated in 0.1 *N* sulphuric acid at 30° C for about 20 days, when they were fully infective (3). Storage either before or after embryonation was at 4° C in 0.1 *N* sulphuric acid. In preparation for hatching 1 ml of this suspension, equivalent to 1 g (3×10^6 eggs) of uterine contents was neutralized with 0.5 *M* phosphate buffer, pH 7.0, washed three times by centrifugation with distilled water, and diluted to 10 ml. This suspension contained about 2% of hatched larvae, which increased gradually upon storage for 1 week at 4° C to 15%. For this reason it was used within 24 hours. Such background hatching occurred only in neutral aqueous suspensions, and never in acid suspensions.

The chitinous shell was removed when necessary by suspending the eggs in sodium hypochlorite solution (fresh undiluted household bleaching solution containing about 6% available chlorine was best for this purpose) and shaking for about 4 hours at 35° C. Disappearance of the shell was complete when a drop of the suspension heated at 80° C and examined under the microscope contained only free larvae and globules of the melted vitelline membrane. The suspension was then washed immediately by centrifugation, dialyzed at 4° C for 24 hours, and used experimentally within 12 hours. Background hatching was about 10%.

Hatching Media

To 0.5 ml of egg suspension in a 15 ml Warburg flask was added 1.0 ml of 0.25 *M* sodium chloride containing 0.0025% Tween 80 and 0.1 *M* reducing agent, and 1.0 ml of pregassed 0.1 *M* sodium bicarbonate. The flask was placed on its manometer immediately and gassed for 15 minutes at 38° C with N_2–CO_2 (95–5), after which the taps were closed and incubation continued as desired. As each flask was removed from the bath its contents were chilled to 0° C and the extent of hatching determined within an hour or two.

In preparing the hatching medium certain precautions were necessary to ensure satisfactory results. The reducing agent was added *before* the bicarbonate solution, because preneutralized sodium hydrosulphite, cysteine hydrochloride, sodium bisulphite, and sulphur dioxide were ineffective hatching stimulants. Sodium hydrosulphite was freshly prepared in a nitrogen atmosphere in oxygen-free water, and cysteine sodium salt and cysteine hydrochloride were dissolved only in triple glass-distilled water. Sulphur dioxide, which was used routinely, was prepared by dilution from a stable, saturated solution stored at 4° C. Under the conditions employed, the pH of the medium before gassing was approximately 6.0 and after gassing at 38° C was 7.0 to 7.1. When the concentration of reducing agent was varied the concentration of bicarbonate was also changed empirically to maintain this final pH.

The extent of hatching was determined by counting a total of 300 to 500 unhatched embryonated eggs and hatched larvae in a suitably diluted drop. A

hatched larva was defined as one which was completely free from the egg, or which had broken through the vitelline membrane bubble and was therefore in direct contact with the medium. In two experiments counts were made on four identical egg samples in which the average hatch was 64% and 74% respectively. The coefficients of variation were 2.4 and 2.6, indicating that the precision of counting was adequate.

Results

The Hatching Curve

Five large batches of infective eggs prepared from different lots of *Ascaris* females collected over a period of 18 months responded almost identically to the hatching stimulus, i.e., 80 to 95% hatching occurred during 3 hours of continuous stimulation at 38° C in the medium described in Fig. 1. Most of the hatching took place in the interval between 30 and 75 minutes. The larvae appeared to be unharmed by any of the media used, for they were always alive several hours after hatching. Eggs from which the chitinous shell had been removed by treatment with sodium hypochlorite hatched much faster than those in which this shell was present (Fig. 1). The digestion of a hole in the shell was, therefore, a rate-limiting step in the enzymatic response to stimulation.

Age of Eggs and Conditions of Embryonation

Storage of eggs for 10 months at 4° C in 0.1 *N* sulphuric acid either before or after embryonation caused only a small decrease both in rate and extent of

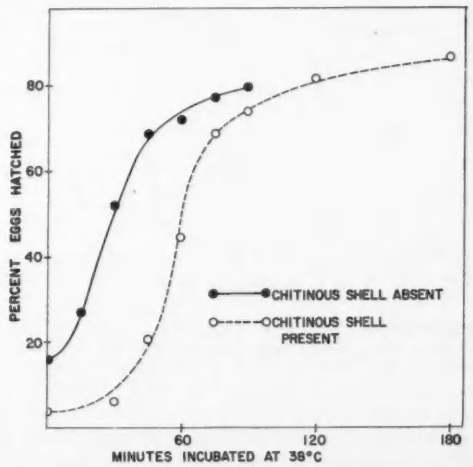

FIG. 1. Hatching of infective *Ascaris* eggs in the presence and absence of the chitinous shell. One milliliter of 0.1 *M* sulphur dioxide solution was added to 0.5 ml of egg suspension (1.5×10^6 eggs) in a series of Warburg flasks. To this mixture was added 1 ml of pregassed solution of 0.25 *M* sodium chloride containing 0.1 *M* sodium bicarbonate and 0.0025% Tween 80. The flasks were gassed with N_2–CO_2 (95–5) at 38° C for 15 minutes, then sealed (zero time), and shaken continuously for periods up to 3 hours.

hatching. In one batch of infective eggs which had been refrigerated for 2 years hatching decreased from 77% to 47%. However, if embryonation was carried out in 1% formalin or in 2% sodium dichromate, hatching in 3 hours was reduced to 25% and 2%, respectively. Possibly these reagents, which like dilute acid are excellent inhibitors of microbial growth, reacted chemically with the shell to make it resistant to digestion by chitinase or other enzymes. In all of these solutions embryonation itself appeared to be normal.

Effect of Embryonic Age

When incubated at 30° C, *Ascaris* eggs develop vermiform motile embryos after 9 days. According to Stoll (18), however, they become infective only after twice this time, presumably because the embryos must be given time to molt once (1). Rogers (13) reported that in two batches of eggs the in vitro hatching could be induced only after 19 and 20 days of embryonation, respectively, thus pointing to a correlation between hatchability and infectivity. This relationship was examined in more detail in an experiment in which samples were withdrawn from a batch of embryonating eggs at 2-hour intervals between the 16th and 21st day of incubation at 30° C. Each sample was refrigerated until all were collected, after which a hatching stimulus was applied

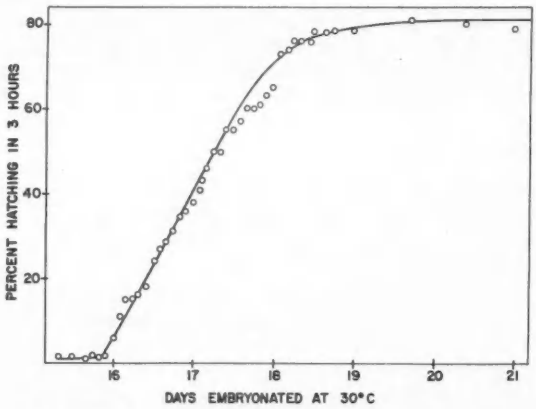

FIG. 2. Development of hatchability as *Ascaris* eggs reach the infective stage. Small samples were removed every 2 hours (beginning on the 16th day) from a large batch of eggs which was shaken continuously at 30° C. Hatchability was determined as described in Fig. 1.

as described in Fig. 1. From the results (Fig. 2) it is apparent that after 3 hours stimulation at 38° C no hatching was induced in eggs embryonated for less than 16 days. During the 17th and 18th days the extent of hatching increased linearly to a figure near the maximum of 80%, which was observed after 19 days. The results were therefore in general agreement with those of Rogers (13) although in this experiment the response to stimulation appeared earlier, and took longer to become maximal.

Influence of Temperature

Specific temperatures were found to be an essential part of the hatching stimulus. Hatching was determined after 60 minutes, i.e., the time at which the rate was maximal at 38° C (Fig. 1) in a series of experiments in which temperatures varied from 30 to 46° C. Little or no hatching was observed below 37° C (Fig. 3). Higher temperatures eliminated hatching, owing to death of the embryos. Over longer periods, some hatching took place at lower temperatures. Thus, after 3 hours at 34° C, 22% of the eggs hatched. At 33° C, 17 hours were required to achieve the same result. Clearly, the optimal temperature for embryonation (30° C) is completely unsuitable for hatching.

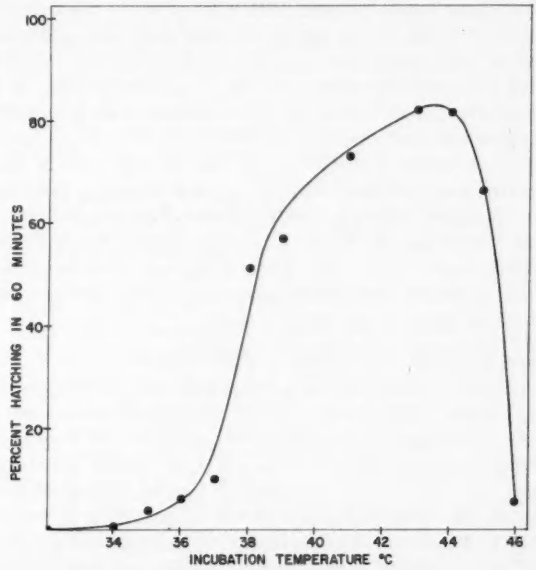

FIG. 3. Influence of temperature on hatchability. Each point on the curve was obtained from a different experiment, as only one variation in temperature could be employed in each experiment. Hatching in controls (60 minutes at 38° C) remained the same throughout the series. Hatching medium as described in Fig. 1.

Reducing Agents

At 38° C in bicarbonate – 5% carbon dioxide buffer (pH 6.5 to 8.0) about 20% of infective eggs hatched. In 0.02 *M* bicarbonate alone (pH 8.4) no hatching occurred. Free carbon dioxide therefore appeared to be a necessary component of the stimulus. Addition of 0.04 *M* cysteine hydrochloride or sodium hydrosulphite (dithionite) greatly enhanced hatching, in confirmation of Rogers (13). However, the instability of these reagents in solution produced inconsistent results unless considerable care was taken in their preparation, and a search for equally effective but stable reagents led to the discovery of sulphur dioxide, sodium bisulphite, or glutathione as useful adjuncts to the

hatching medium. Of these three, sulphur dioxide was adopted for routine use. In general, the gas atmosphere used was nitrogen – carbon dioxide, 95–5. However, this was necessary only in the presence of cysteine or hydrosulphite, the other reagents being stable in nitrogen – oxygen – carbon dioxide (75–20–5) and hatching being equally satisfactory. An atmosphere of oxygen – carbon dioxide (95–5) completely inhibited hatching.

All the reducing reagents mentioned formed acid solutions which were subsequently neutralized to pH 7.0 by means of appropriate concentrations of gassed bicarbonate buffer. It was found that brief exposure of the eggs to slight acidity, approximating pH 6.0 or lower, was essential for hatching, and it was for this reason that emphasis was given to the order of addition of reagents to the hatching medium (see Methods). Preliminary neutralization of the reducing agents to pH 7.0, or use of an essentially neutral compound such as cysteine sodium salt instead of cysteine hydrochloride, inhibited hatching almost completely. No explanation for this phenomenon can be suggested.

Provided that the concentration of bicarbonate was adjusted to produce pH 7.0 in the gassed medium, variations in the concentration of sulphur dioxide or sodium bisulphite between the limits of 0.01 *M* and 0.08 *M* did not greatly affect the extent of hatching in 3 hours. Conversely, in a medium containing 0.04 *M* sulphur dioxide, varying the number of eggs per flask from 0.5 to 12×10^5 did not influence hatching. Taken together, these experiments suggested, but did not prove, that the action of this reducing agent was related to the poising of an oxidation–reduction potential in the medium rather than to chemical reaction with egg material.

Effects of Sodium Chloride and Surface-Active Agents

Rogers (13, 14) stated that hatching was improved in the presence of sodium or potassium chloride and a surface-active agent such as sodium taurocholate or Tween 80. In our experiments no beneficial effects of inorganic salts were observed, although sodium chloride was retained in the hatching medium in order to increase the viability of the hatched larvae. Tween 80 had little effect upon hatching of the freshly embryonated eggs. After 6 weeks' storage of eggs in acid at 4° C, however, the hatching medium was somewhat less effective when Tween 80 was omitted (Fig. 4). As it was convenient to store eggs over considerable periods of time, this compound was routinely incorporated in the medium. Ordinarily, infective eggs remained in the hatching medium throughout the experiments. In two experiments this procedure was modified so that the medium was removed after a predetermined exposure of the eggs to it, and incubation was continued in water, the total exposure to medium plus water being in all cases 3 hours. The results were then compared with those obtained in the usual way by continuous exposure to hatching media (Table I). Best results were obtained when the stimulus supplied by the hatching medium was applied for at least 90 minutes. However, exposure for as little as 40 minutes was sufficient to produce continued hatching when the eggs were removed into water. It was also observed in a number of experiments that after partial stimulation hatching continued slowly for a period of 24 hours or more at 4° C, even although it had been clearly established (Fig. 2) that the stimulus itself was ineffective below 33° C. It was concluded, therefore,

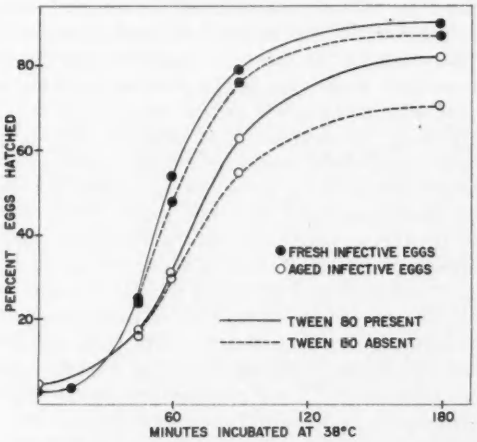

FIG. 4. Effect of Tween 80 on hatching. Fresh infective eggs were those placed in hatching medium after 20 days embryonation at 30° C. Aged infective eggs were stored an additional 6 weeks in 0.1 *N* sulphuric acid at 4° C before hatching. Final concentration of Tween 80, 0.001%. Hatching medium as in Fig. 1.

TABLE I

Effect of duration of the stimulus upon hatching

No. minutes stimulated	% hatching	
	During stimulation	In 3 hours
0	3	5
20	5	10
40	12	29
60	25	51
90	61	83
180	80	—

NOTE: At zero time and intervals thereafter, as indicated in the table, 1 ml of the egg suspension in hatching medium (see Fig. 1) which had been gassed for the preceding 15 minutes was placed in 10 ml of water at 0° C and centrifuged. After washing twice with 1 ml of cold water the eggs were placed in water at 38° C and left until the total elapsed time at 38° C (including the stimulation time) was 180 minutes. Each figure is an average from two experiments.

that stimulus and response were separate phenomena, and that the response could continue in the absence of the stimulus.

Permeability Changes during Stimulation

If chitinase and other enzymes are liberated within the egg as a result of stimulation, it is necessary to account for their passage through the highly impermeable vitelline membrane (4) in order that the chitinous shell may be digested.

Although the vitelline membrane itself was not visibly changed as a result

of exposure of the eggs to hatching medium, it seemed possible nevertheless that changes had occurred which led to increased permeability. If, as would be necessary, these changes preceded the migration of enzymes through the membrane, then smaller molecules might also be expected to leak from the egg during stimulation, and at a rate greater than the actual rate of hatching.

Fairbairn and Passey (6) reported that infective eggs contained high concentrations of the disaccharide trehalose, which occurred almost exclusively in the perivitelline fluids bathing the embryo, and which could be extracted quantitatively by brief treatment with hot water. Other carbohydrates such as glycogen were unable to pass through the chitinous shell. In preliminary experiments it was established that no leakage of carbohydrate occurred in unstimulated eggs incubated at 38° C, and that as a result of stimulation the alkali-stable, 50% ethanol-soluble carbohydrate (trehalose) was not hydrolyzed or otherwise degraded. Several experiments were then performed in which the eggs were exposed to the hatching stimulus for variable periods of time, as in Table I, then washed at 0° C and refrigerated for 24 hours. This refrigeration period was necessary because diffusion of trehalose from the eggs, either before or after hatching, was slow. The percentage of hatching was then determined, and compared with the anthrone-sensitive carbohydrate (trehalose) which had diffused from the eggs, whether hatched or unhatched. This carbohydrate was calculated as a percentage of the total carbohydrate extracted with hot water from unstimulated eggs. In every experiment (Fig. 5) trehalose appeared in the hatching medium before appreciable hatching had occurred and continued at a high level once hatching had commenced. Although more detailed investigation is needed, the experiments suggested that one of the first changes occurring during hatching was the appearance of increased per-

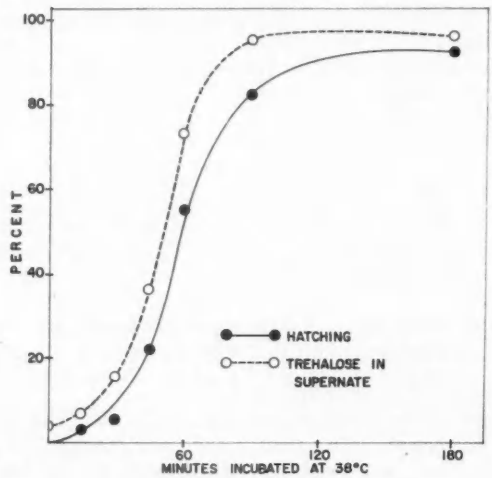

FIG. 5. Leakage of trehalose from stimulated eggs. For description of experiment, see the text. Hatching medium as in Fig. 1.

meability in the vitelline membrane. Wilson (19) has shown that permeability changes are also important in the spontaneous hatching of a strongyloid egg (*Trichostrongylus retortaeformis*).

Discussion

The in vitro stimulus leading to hatching of *Ascaris* eggs consists of (1) a suitable temperature, similar to that of homoiothermic animals, (2) the presence of non-specific reducing agents, (3) free carbon dioxide, (4) a pH near neutrality. Because these are conditions existing in the small intestine of the feeding host, where in vivo hatching occurs (2, 7, 12), and because morphological changes in vivo and in vitro are identical, it is reasonable to conclude that the in vitro stimulus corresponds fairly closely to that provided by the host. Additional evidence is provided by the observed effects of surface active agents (taurocholate, Tween 80) and the inability of either the natural or experimental stimulus to promote hatching in eggs which are incompletely embryonated (Figs. 2, 4, and Rogers (13)). The nature of the naturally occurring reducing substances is not known, although the digesting intestine can be expected to contain considerable amounts of sulphydryl compounds, of which both cysteine and glutathione are effective in vitro.

Much remains to be done before the action of the stimulus can be understood. It is well known, for example, that the vitelline membrane of *Ascaris* eggs is truly semipermeable, in the sense that only gases can pass through it (4). Thus, it is difficult to explain how hydrogen ion concentration and sulphydryl compounds can fulfill their obvious function unless the presence of carbon dioxide alone leads to an increased permeability of the membrane. Preliminary experiments have not supported this idea, although there is some evidence, based on leakage of trehalose during stimulation (Fig. 5) that the *whole* stimulus leads to the development of permeability during early stages of the response. As to the site within the egg at which the stimulus is received, there is also no information.

In our experiments, and those of Rogers (13) the stimulus was always applied to decoated eggs, as those retaining the outer layer of the egg shell are sticky and hard to work with. During normal passage of the freshly laid egg through the intestine, this outer layer hardens, becomes chemically resistant (9), and persists throughout embryonation. A completely satisfying picture of in vitro hatching would, therefore, require that the stimulus be shown to lead to hatching of such eggs. This also remains to be shown.

It is possible to envisage conditions, for example in sewage, or in flooded soils, which would provide suitable concentrations of hydrogen ions, reducing agents, and carbon dioxide to promote in vitro hatching. If this were so, the one component of the stimulus peculiar to the hosts would be the elevated temperature in the absence of which no hatching can take place. Given this, the stimulus is seen to be common to many mammals, and it is, of course, well-known that abortive *Ascaris* infections occur widely in different hosts. Apparently, a much higher degree of host specificity applies to later stages of development than to hatching itself.

From a practical viewpoint, it is pertinent to emphasize that hatching, and hence infectivity, is affected by the conditions of embryonation. Formalin, and

potassium dichromate solutions, have been used very generally as media for the embryonation of nematode eggs, because they are effective germicides but do not hinder development. If, in nematodes besides *Ascaris* these disinfectant solutions also make the eggs difficult or impossible to hatch, they are obviously unsuitable for use in the study of infectivity and related biological problems. Lund and Burtner (8) have recently shown that this is true in the case of *Heterakis gallinae* eggs which had been embryonated in dichromate solution.

The eggs of two other ascarids (*Toxocara mystax*, *Ascaridia galli*) hatch under the influence of a similar stimulus, which also leads to exsheathment of several infective trichostrongyle larvae (14–17). It appears, therefore, that these organisms have tended to adapt to common conditions existing in the intestinal tract of many homoiothermic animals (5).

Acknowledgment

The author is indebted to Tibor Fuleki for skilled technical assistance.

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PARATYLENCHUS TENUICAUDATUS N. SP. (NEMATODA: CRICONEMATIDAE)[1]

L. Y. Wu

Abstract

Paratylenchus tenuicaudatus n. sp. was collected in Ontario, Canada, from soil about roots of clover (*Trifolium repens* and *T. pratense*), of alfalfa (*Medicago sativa*), of maple tree (*Acer saccharum*), and from grass sod, during 1958 to 1960.

This species has characters close to *Paratylenchus hamatus*, *P. elachistus*, and *P. nanus*, but differs from these species in having a long slender tail, in having the vulva in a more forward position, in having a more massive basal bulb of the esophagus, and in the fact that it is a considerably larger species.

Female

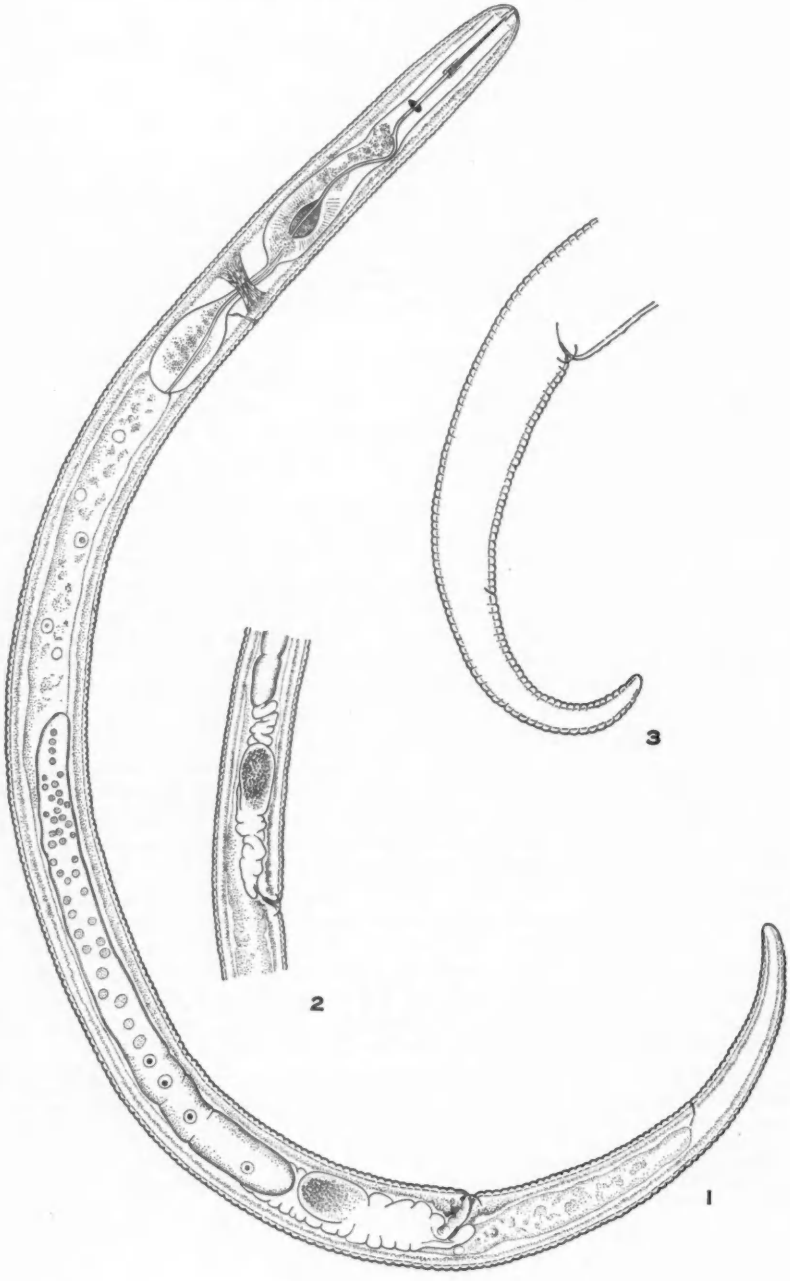
Body of heat-killed specimens slightly curved ventrally to C-shaped. Body cuticle marked by coarse annulations, about 1.5 to 2 μ wide. Lateral field about one-quarter body width, with four incisures, equally spaced. Phasmids not determined. Excretory pore generally at anterior level to middle of basal bulb.

Spear slender, 25.1 to 31.5 μ (mean = 28.1 $\pm$ 0.2). Spear knobs probably equal in size but outlines of knobs frequently give the appearance of being slightly asymmetrical. Esophagus 0.097 to 0.124 mm long (mean = 0.111 $\pm$ 0.001). Both esophageal bulbs massive, median bulb club-shaped, 9 to 15.3 μ in diameter; basal bulb oval to oblong, 10 to 15.7 μ in diameter, usually slightly larger than median bulb, but in some cases vice versa, or equal. Dorsal gland orifice 6 to 9 μ behind base of spear. Contour of esophagus near area of dorsal gland orifice very often disturbed because of the profound excretory accumulation. Esophagus in front of base of spear uniform in width. Isthmus slender and long with nerve ring about middle.

Ovary single, anterior, 0.089 to 0.342 mm long (mean = 0.17), longer in older specimens, the longest ovary extending to level 8 μ behind basal bulb. Ovary outstretched. (In one specimen the ovary was reflexed.) Oöcytes in single row at distal end, double or irregularly double at proximal end, except a few cells in single row at the tip of ovary. Eggs, in uteri, 37 $\times$ 20 and 47 $\times$ 18.5 μ. Uterus and oviduct more slender in young than in old females (Fig. 2). Spermatheca present, appears to be a side pouch, oval to oblong in young specimens. Its shape and size changed in old females, being much larger and more or less square or triangular. A very small sac present posterior to vagina, probably the vestige of postvulval uterine sac. Vulva membrane present. Body immediately behind vulva reduced gradually in young females, but reduced abruptly in old specimens.

Tail slender, 0.037 to 0.058 mm long (mean = 0.045 $\pm$ 0.001), 3.5 to 5 times body width at anal region. Tip of tail conically rounded. Tail in old specimens very often bent into hook-like curve (Fig. 3).

[1]Manuscript received January 6, 1961.
Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.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Number of postvulval annules about 55 to 70; number between vulva and anus 28 to 34, annules coarse, average 1.5 to 2 μ wide; number of annules on tail about 26 to 35, those near the terminus often could not be accurately determined.

Body length 0.381 to 0.600 mm (58 females), with mean = 0.468 $\pm$ 0.007 mm; body width 0.014 to 0.026 mm; distance between vulva and anus = 0.04 to 0.066 with mean = 0.051 $\pm$ 0.002; a = 18.5 to 30.6; b = 3.4 to 5.1; c = 9.0 to 13.1; $V\%$ = $^{20.1-61.7}$ 77.6–81.6.

Male

Unknown.

Diagnosis

This species is close to *P. hamatus*, *P. elachistus*, and *P. nanus* in having the lip region rounded and smooth, and in the length of the spear, but distinguishes itself from all three species in having a long slender tail, more forward position of the vulva, more massive basal bulb, and in being a much larger species.

Type specimens.—Holotype ♀, collection No. 1566, collected May 1959; 57 paratypes, collection Nos. 1379, 1382, 1388, 1403, 1890, 1894, 1979; all in Canadian National Collection of Nematodes, Nematology Section, Entomology Research Institute, Ottawa, Ontario.

Type host.—Soil around roots of red clover (*Trifolium pratense* L.).

Type locality.—North Gower, Ontario.

Host range.—In soil around roots of *Trifolium pratense* L., *T. repens* L., *Acer saccharum* Marsh, *Medicago sativa* L., and grass sod.

Distribution.—North Gower, City View, Perth, Fallowfield, Smiths Falls, Gillies' Corners, Prescott, in Ontario, Canada.

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Figs. 1–3. *Paratylenchus tenuicaudatus* n. sp. 1. Female. 2. Portion of a young female, showing part of ovary, oviduct, spermatheca, and uterus. 3. Caudal end of an old female.

OBSERVATIONS ON THE LIFE CYCLE OF CAPILLARIA HEPATICA (BANCROFT, 1893) WITH A DESCRIPTION OF THE ADULT[1]

K. A. WRIGHT[2]

Abstract

The morphology of adult *Capillaria hepatica* (Bancroft, 1893) Travassos, 1915 is described in detail from a collection of two entire specimens and 16 fragments. Experiments are reported which indicate that eggs of *C. hepatica* obtained from *Peromyscus maniculatus* in Algonquin Park, Ontario, when embryonated, unembryonated, or in various stages of development, are resistant to the low temperatures they might encounter during northern winters. The morphology of the larval stages is also described and evidence is cited indicating a direct larval migration to the liver of the host probably through the hepatic portal system.

Introduction

Capillaria hepatica (Bancroft, 1893) has been reported from the liver of a wide range of mammalian hosts including man (10). Despite its rather common occurrence in rodents, its morphology has not been well described. The most complete description of this species is that given by Baylis (3), which was based on a study of fragments of males and females. Entire specimens are difficult to obtain since the worms become tightly coiled within the liver of the host. During the present study, two entire worms as well as 16 fragments were studied.

The results of experiments by Luttermoser (11) on the ability of the eggs of *C. hepatica* to withstand low temperatures have been interpreted by Pavlov to mean that the eggs of this species could not remain viable throughout cold winters (15). In view of the mode of transmission of *C. hepatica* in a population of *Peromyscus maniculatus* in Algonquin Park, Ontario (7), this seemed unlikely. Hence, experiments were conducted to determine the effects of low and high temperatures upon the eggs of *C. hepatica* obtained from *P. maniculatus* of Algonquin Park.

Little is known of the morphology of the larval stages of this parasite, although Fülleborn (9) and Luttermoser (12) measured larvae of various ages recovered from the livers of rats. The sequence of larval molts is unknown.

The route of larval migration after hatching of the larvae in the caecum of the host was investigated by Fülleborn (9), Nishigori (13), and Suda (16). These authors concluded that the larvae migrated directly by way of the hepatic portal system to the liver of the host. However, the work of Luttermoser (12) might indicate a more indirect route, since he states that larvae began to penetrate the wall of the caecum as early as 6 hours after eggs were fed to rats. Yet, the earliest record of larvae occurring in the liver of the host is that

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of Fülleborn, who recovered larvae 42 hours postfeeding. Larvae might be expected to reach the liver in less than 36 hours if a direct migration through the hepatic portal system occurred. In the present study experiments were designed to recover larvae of various ages for morphological study, and to elucidate the route by which the larvae migrate to the liver.

Materials and Methods

Experimental infections were established with eggs taken from a *Peromyscus maniculatus gracilis* (LeConte) trapped in Algonquin Park, Ontario. Twenty white mice were fed these eggs and 5 weeks later were killed, their livers minced in a Waring blendor, the sediment ground more finely with a tissue grinder, and the mixture repeatedly sedimented and decanted in tap water until the supernatant was clear. These eggs, in a minimum of tissue debris, constituted the basic egg supply. They were stored in a minimum volume of 0.5% formalin to decrease bacterial growth, and the vials were aerated two or three times each week. Additional eggs were collected, when required, by the same method from experimental infections.

Embryonation of the eggs after exposure to various temperatures was determined by counting the number of eggs in various developmental stages in the first 100 eggs seen on a slide. This was repeated, the first value being taken unless the values differed by more than 3%, in which case five or six slides were counted and the results averaged.

Embryonated eggs were fed to white mice in order to recover adult and larval specimens. This was done by pipetting a large number of eggs into the mouth of each mouse. By this means a large number of eggs could be administered at a known time.

Larvae were recovered from the liver of these mice by the use of a Baermann apparatus. The blood stream was examined for larvae by a modification of the perfusion technique of Yolles *et al.* (20).

Sections of adult worms were cut 8 μ thick and stained with Harris' haematoxylin and eosin.

Morphology of Adult *Capillaria hepatica*

Trichuridae Railliet, 1915; Capillariinae Railliet, 1915; *Capillaria* Zeder, 1800. Long, filiform nematodes tapering gradually from a maximum diameter near the middle of the body to a very fine anterior extremity; only slightly tapering posteriorly.* Cuticle with fine transverse striations. Two conspicuous, lateral bacillary bands extend the entire length of the body. Oral opening a dorsoventral slit followed by a shallow, dorsoventrally ovoid buccal cavity. Cephalic papillae consisting of inner circle of six and external circle of eight papillae. Lateral pore-like amphids present (Fig. 1). Oesophagus consisting of thin, muscular, anterior portion and posterior portion surrounded by variable number (21–32) of stichocytes. Anterior part of oesophagus 1/8 to 1/13 length of posterior part. Nerve ring surrounding anterior part of oesophagus 1/10 distance from anterior extremity (Fig. 3). A single ventral cell at juncture of

*Fixation often results in a marked narrowing of the body from the base of the oesophagus to the posterior end of the vagina.

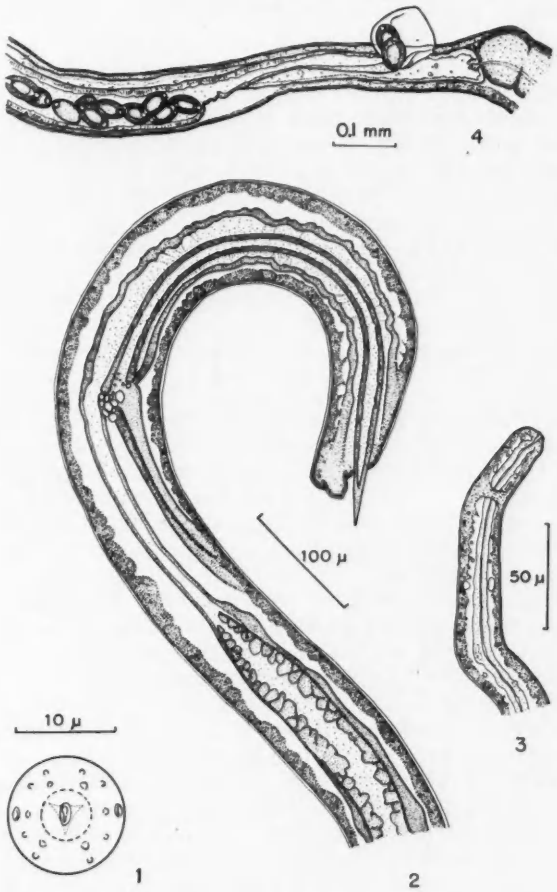


FIG. 1. *En face* view of adult female.
FIG. 2. Posterior end of adult male, drawn from living specimen.
FIG. 3. Anterior end of adult female, drawn from living specimen.
FIG. 4. Portion of adult female showing vulvar appendage.

oesophagus and intestine. Oesophageal–intestinal valve composed of six cells projecting into lumen of intestine.

Female:* Total length 52 mm (69). Maximum width 0.11 mm (0.89–0.16). Nerve ring 0.048 mm (0.038–0.084) from anterior extremity. Anterior oesophagus 0.38 mm (0.32–0.68) in length. Posterior oesophagus 5.4 mm

*First measurements given are from a single complete worm USNM No. 39297; measurements given in parentheses are the range derived from the study of a second complete worm USNM No. 39298 and 11 fragments. Both complete worms were obtained from the body cavity of an experimentally infected *Peromyscus leucopus*.

(5.2–7.8) in length. Vulva 0.11 mm (0.11–0.17) behind oesophagus, with bell-shaped cuticular appendage (Fig. 4). Vagina 0.17 mm in length containing single row of eggs. Uterus coiled, 12 mm in length connected to ovary by oviduct 1.5 mm in length. Single hologonic ovary 34 mm in length ending ventrally to rectum. Anus subterminal. In addition to lateral bands, a narrow bacillary band present in hypodermis of ventral line. Eggs doliiform, with radially striated protein coat, 49–66 μ (av. = 54 μ) by 28–36 μ (av. = 31 μ).

Male:* Maximum width 0.08–0.074 mm. Single hologonic testis extending posteriorly from 0.08 mm behind base of oesophagus and opening into cloaca at same level as intestine. Single tubular spicule 0.46–0.54 mm in length, 0.009–0.010 mm wide, dilating to 0.012 mm near the pointed tip. Eversible spicule sheath without spines. Anus subterminal; caudal extremity bearing two large lateroventral lobes (Fig. 2).

Four parts of this description are worthy of further comment.

Bacillary Bands

Baylis (3) first recorded the presence of bacillary bands on *C. hepatica*. He stated that they ". . . are apparently situated dorsally and ventrally . . . and . . . extend (at least in the female) throughout the greater part of the body." Freitas and Lent (8) observed but did not give the positions of the bacillary bands. In contrast to Baylis's description, Chitwood and Chitwood (4) stated that the bacillary bands of *Trichuris* and related forms are located in the hypodermis of the lateral line.

Cross sections made in the present study through the region of the vulva of *C. hepatica* revealed that the bacillary bands are indeed lateral in position (Fig. 5). A third narrower and less conspicuous band was found in the ventral line of the female. The bands, extending continuously from the extreme anterior end to the tail, consist of uninucleate cells, embedded in the hypodermis. The cells are spherical to columnar, with a raised "neck" which opens through a pore in the cuticle (Figs. 6 to 8). In living material, striations were readily seen radiating into the cell from the "neck" region. Neutral red stained the neck region in living material. When seen in superficial view, the cells present a hexagonal outline if many are present, or a nearly spherical outline if few are present. The pore appears as a bright refractive spot or ring (Fig. 7). The depth of the band is greatest in the oesophageal region where the cells are columnar. The lateral bands are up to 35 to 40 cells in width, covering more than half of the circumference of the body, while the ventral band in the female is only five to six cells in width.

The function of these cells is unknown. Chitwood and Chitwood (4) regarded them as homologous to the lateral hypodermal glands found in free-living marine, fresh water, and soil-inhabiting members of the orders Enoplida and Chromodorida.

Spicule Sheath

Spines were described on the proximal portion of the spicule sheath of specimens of *C. hepatica* by Freitas and Lent (8). Although, in the present study, male fragments were studied alive, and fixed and cleared in both glycerin

*Measurements given are the ranges derived from the study of five fragments.

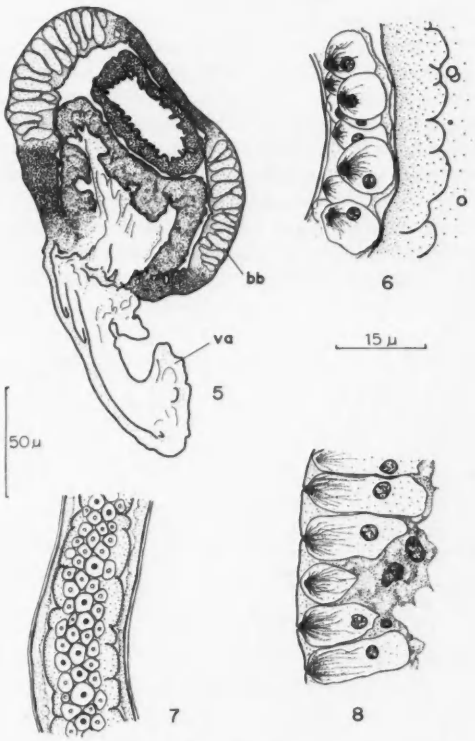


FIG. 5. Cross section through region of vulva. *bb* = bacillary band; *va* = vulvar appendage.
FIG. 6. Cells of bacillary band as seen in living material stained with neutral red; drawn free-hand while living.
FIG. 7. Surface view of lateral bacillary band in region of oesophagus.
FIG. 8. Cells of bacillary band as seen in longitudinal section, stained with haematoxylin and eosin.

and lactophenol, no such spines were seen. Through the courtesy of Dr. Teixeira de Freitas of the Instituto Oswaldo Cruz, the author was able to study the male fragment illustrated by Freitas and Lent. In this specimen irregularly distributed, minute, refractive spots as well as a more regular pattern of short transverse bars could be seen on the sheath in the region immediately outside the body. Similar spots were seen on the cuticle of the body itself. It was concluded that these were artifacts. No spines could be seen on the sheath within the body.

Cephalic Papillae

The arrangement of the cephalic papillae was determined by studying seven *en face* preparations following the method of Anderson (1). The entire complement of cephalic papillae was not distinguished in any one specimen. However,

external pore-like amphids and six papillae, apparently of the inner circle, were seen in most preparations, and in some the grouping of what may be the lateroventral and ventroventral, or the laterodorsal and dorsodorsal papillae of the external circle were seen. Assuming that the papillae of the external circle are symmetrically distributed, there probably is a complete inner circle of six papillae and an external circle of eight papillae (Fig. 1). The anterior extremity of *C. hepatica* is extremely minute, and the cephalic papillae much reduced in size; therefore these conclusions will probably need confirmation.

According to Chitwood and Chitwood (4), the only other species of the Trichuroidea in which cephalic papillae have been studied is *Trichuris suis*. In this species only six papillae, presumably of the external circle, were observed.

Oesophageal Ventral Cell

Two ventrolateral cells are described at the base of the stichosome of most species of *Capillaria*. These cells were described as glandular by Eberth (5, 6), who found that they connected with the lumen of the oesophagus. Skrjabin *et al.* (15) included a figure of *C. hepatica* by Pavlov (1955) which indicates two such cells. However, neither Baylis (3) nor Freitas and Lent (8) recorded the presence of these cells in *C. hepatica*.

Only one ventrolateral cell was found in sectioned material and in whole specimens examined in the present study. This was verified in the study of larval stages. In some specimens of second- and third-stage larvae when viewed ventrally, another cell was seen projecting to either side of the oesophagus (Figs. 12 and 14). This cell, which extended dorsally across the oesophageal–intestinal juncture, was interpreted as a coelomocyte, not related to the ventral cell. No connection between the ventral cell and the lumen of the oesophagus was seen.

Embryonation and the Effects of Temperature on the Viability of the Eggs

The eggs of *C. hepatica* are uncleaved *in utero*, but may divide to the four-, or rarely, to the eight-celled stage after being deposited in the liver of the host. Further cleavage does not take place until the eggs are released from the liver tissue.

Bancroft (2) found that vermiform larvae had developed 5 months after eggs were dissected from the liver of rats and kept at room temperature. Shorb (14) found that after 25 days eggs kept at 22° and 30° C were only partly embryonated, while most of the eggs kept at 37.5° C were fully embryonated. After 42 days, larvae were present in eggs kept at both 22° and 30° C, while the larvae in most of the eggs at 37.5° C had degenerated. Luttermoser (11) was able to embryonate eggs at room temperatures in fluid cultures, or in moist fecal pellets within about 6 weeks, and considered that all the eggs were fully embryonated within 8 weeks. He showed that moisture, temperature, and oxygen tension were critical factors. In his studies, eggs remained alive for 1 to 2 weeks when exposed at room temperature to relative humidities less than 50%. Unembryonated eggs were killed by exposure to temperatures of −1° to 5° C for 7 days, while similar eggs exposed to temperatures of −7° to 1° C

were killed after 3 days' exposure. The development of larvae was greatly reduced when embryonated eggs were exposed for 4 days to 35° C, and exposure to this temperature for 7 days or more was lethal. This disagrees with the work of Shorb (14). However, some of the eggs kept by Luttermoser under reduced oxygen tension at 35° C remained alive for 2 weeks. Accordingly, he suggested that the lethal metabolic processes, active at 35° C, are suppressed in the host by lack of oxygen, and that this might explain why eggs can remain viable within the host for as long as 8 weeks.

In the present study some observations were made on the viability of the eggs within the host, and under laboratory conditions. Eggs, separated from the liver of a mouse infected 681 days previously, embryonated and produced infections. Since female worms were found to live only 60 days, the eggs must remain viable within the host for a greater period of time than that reported by Luttermoser (8 months). Also, eggs kept in unfrozen liver tissue in a refrigerator for 750 days subsequently embryonated and produced infections.

Occasionally eggs were found in abnormal sites within the host. In some mice fed excessively large doses of eggs, living, adult worms were found attached by their anterior ends to the liver but lying free in the body cavity. Usually such a massive infection killed the host, but occasionally the host lived long enough for eggs to be deposited in the body cavity. Forty-seven per cent of such eggs collected from one mouse developed infective larvae within 8 weeks at room temperature. Egg deposition in the body cavity was prevented in most instances by a heavy growth of host connective tissue around the worm. Some of the eggs produced by worms lying in the body cavity had abnormally thick shells. These eggs, as well as eggs with an abnormally thin shell, were sometimes found in otherwise normal infections of the liver. Both types of eggs were found to embryonate at room temperature.

Considering the contradictions in the results of previous workers as to the effect on the eggs of high and low temperatures, and considering that *C. hepatica* has been found to be transmitted during the winter in Algonquin Park, Ontario (7), when low temperatures prevail, the effects of these variables were again examined in the present study.

The development of the embryo at room temperature and 37° C is outlined in Table I. At room temperature, fully formed larvae were first found after 5 weeks. Eggs kept at 37° C developed rapidly to the morula stage, but after 4 weeks at this temperature further development was greatly retarded and some eggs were degenerate. No vermiform larvae developed after 10 weeks at 37° C. The eggs were then placed at room temperature, but after a further 5 weeks none had become fully embryonated. These results agree with those of Luttermoser (11).

Eggs were also embryonated at 30° C but a critical account of their rate of development was not kept. It was obvious, however, that within 4 weeks a higher percentage of eggs contained vermiform larvae than eggs kept at room temperature. These larvae did not degenerate if kept at 30° C for as long as 1 month. Embryonated eggs have remained viable at room temperature for 25 months.

Four experiments were carried out to test the viability of eggs exposed to low temperatures during various stages of development.

TABLE I

Embryonation of eggs of *C. hepatica* at room temperature (approximately 20–24° C) and 37° C*

Time in weeks	% uncleaved eggs	% cleavage stages	% morula stages	% tadpole stages	% fully embryonated	% degenerate
0	100 (100)					
1	85 (44)	15 (56)				
2	38 (38)	62 (50)	(12)			
3	36 (16)	16 (50)	43 (34)	5		
4	28 (16)	14 (42)	47 (21)	11		(21)
5	28 (8)	16 (43)	37 (19)	23 (2)	6	(28)
6	20 —	1 —	32 —	31 —	15 —	—
7	12 —	4 —	11 —	13 —	57 —	3 —
8	15 (9)	1 (4)	1 (16)	4 (2)	63 (0)	16 (69)
9	— (10)	— (4)	— (10)	— (5)	— —	— (71)

*First figure in table is for eggs kept at room temperature, figures in parentheses are for eggs kept at 37° C.

Experiment No. 1

Vials of unembryonated eggs were exposed for varying times to three temperatures: series A 4.5 to 13.5° C (av. = 9.0° C), series B −13.0 to −3.0° C (av. = −9.3° C), and series C −17.0 to −12.0° C (av. = −15° C). Two control vials were kept at room temperature (20 to 24° C). A vial was removed from each of series A and B after exposures of 1, 3, 5, 7, 11, 15, and 20 days, and from series C after exposures of 33, 43, and 60 days. The percentage of fully embryonated eggs in each vial was determined after each had been at room temperature for 8 weeks. This percentage in series A varied from 82 to 86% (av. = 84.0), in series B from 48 to 80% (av. = 66.0), in series C from 53 to 62% (av. = 58.0); the control for series A and B had 86% development, and the control for series C had 54%. Eggs from each vial produced infections when fed to mice.

Experiment No. 2

Vials of eggs, fully embryonated at room temperature, were exposed for varying times to three temperatures; series D 9.5 to 13.5° C (av. = 11.7° C), series E −10.0 to −13.0° C (av. = −11.3° C), and series F −14.0 to −16.5° C (av. = −16.3° C). The eggs used in series F were those used in series C of experiment No. 1 which had been allowed to embryonate at room temperature for 8 weeks. Vials were removed from the low temperatures after exposures of 1, 3, 5, 11, 30, 40, and 60 days and samples of eggs were fed immediately to mice. All mice became infected.

Experiment No. 3

Two vials of unembryonated eggs were exposed to a temperature of −40° C, one for 22, and one for 46 hours. They were then brought to room temperature and allowed to embryonate for 8 weeks. At this time, 73% of the eggs exposed for 22 hours, and 45% of the eggs exposed for 46 hours were in the morula stage, while the remainder were degenerate. The eggs were examined 1 month later but no further development had occurred.

Experiment No. 4

Eggs were allowed to develop at room temperature for 2, 3, 4, 5, 6, and 7 weeks, and at the end of these times were exposed to temperatures of −14.0 to −17.0° C (av. = −15.5° C) for 2 weeks. They were then returned to room temperature. The percentage of fully embryonated eggs was determined after all eggs had been at room temperature for a total of 8 weeks, including the period at this temperature before exposure to cold. This percentage varied from 60 to 81% (av. = 70) for the experimental eggs, while 63% of the control eggs, kept throughout at room temperature, were embryonated.

These experiments indicate that eggs of *C. hepatica* taken from mice of Algonquin Park can withstand exposure to temperatures near and below 0° C for considerable periods of time, but cannot withstand the extreme temperature of −40° C for even 1 day. Both unembryonated and completely embryonated eggs survived exposure to −15° C for up to 60 days. Eggs at various stages of their development also survived exposure to this temperature for 2 weeks.

Swales (17) has shown the insulating effect of snow upon both surface and subsurface ground temperatures at Macdonald College, Quebec. During 1

year the ground surface temperature did not fall below 21° F (−5.5° C) and reached this temperature only when all but 1 inch of snow had been removed. If a similar effect may be assumed for snow cover in Algonquin Park, it may be concluded that the eggs of *C. hepatica* from *Peromyscus* in Algonquin Park can survive prolonged exposure to the low temperatures which they might encounter in nature.

The eggs used in this study withstood temperatures considerably lower than those found by Luttermoser to be lethal to eggs of the same species obtained from rats in Baltimore city. Possibly two races of *C. hepatica* exist.

Development of the Larvae

The First-stage Larva

First-stage larvae were obtained from three sources: from eggs by pressing on the cover slip, from the caecum of mice, and from the liver of mice. No larvae were recovered from the blood, lungs, body cavity, spleen, or kidneys of 26 mice examined 1 to 3 days after infection. The route to the liver must be direct rather than circuitous since first-stage larvae were found in both the caecum and liver 52 hours after infection, while no larvae were found in the liver 48 hours after infection. Probably larvae penetrate the hepatic portal system, and are rapidly transported to the liver in the blood.

Larvae hatched mechanically from fully embryonated eggs closely resembled in degree of differentiation larvae recovered within the first 24 hours from the caecum of mice (the early first-stage larva, Fig. 9). Larvae recovered 2 or 3 days after feeding although showing no increase in size, were more highly differentiated (the late first-stage larva, Fig. 10).

In both early and late first-stage larvae the body gradually tapered posteriorly to a subterminal anus. Anteriorly, the body tapered less markedly giving a characteristically blunt "head". A stylet, 6 μ in length, was present (Fig. 11).

In the early first-stage larva the oesophageal region could be distinguished as a clear area in the anterior half of the body. No cellular structure was discerned even after staining in cotton blue, although the lumen of the oesophagus appeared as a fine line extending from the stylet to the beginning of the intestine. The intestine, equally obscure, was distinguished by the presence of closely packed, refractive globules whose size increased posteriorly. The nerve ring was not seen with certainty in any specimen, although a more dense area 8 to 9 μ from the anterior end, seen in larvae stained in cotton blue, may represent this structure. Late first-stage larvae, recovered from the caecum of mice 2 or 3 days after feeding, were more differentiated. The oesophagus was distinguishable, and in some specimens the lumen was enlarged. The limits of the intestine could be clearly seen, and one or two globules only were present posteriorly. Observations of these globules in the intestine of larvae from 4 hours to 3 days of age showed that they disappeared with time. This suggests that they may be food stores. They appear to be utilized in a posteriorly directed gradient during the differentiation of the larva. The first-stage larva may gain all of its nourishment from this source since it appears that the mouth and anus do not open until the late first stage. The stylet was found protruding in only four larvae 2 and 3 days of age. Even in the late first stage, the cuticle did not extend into the rectum.

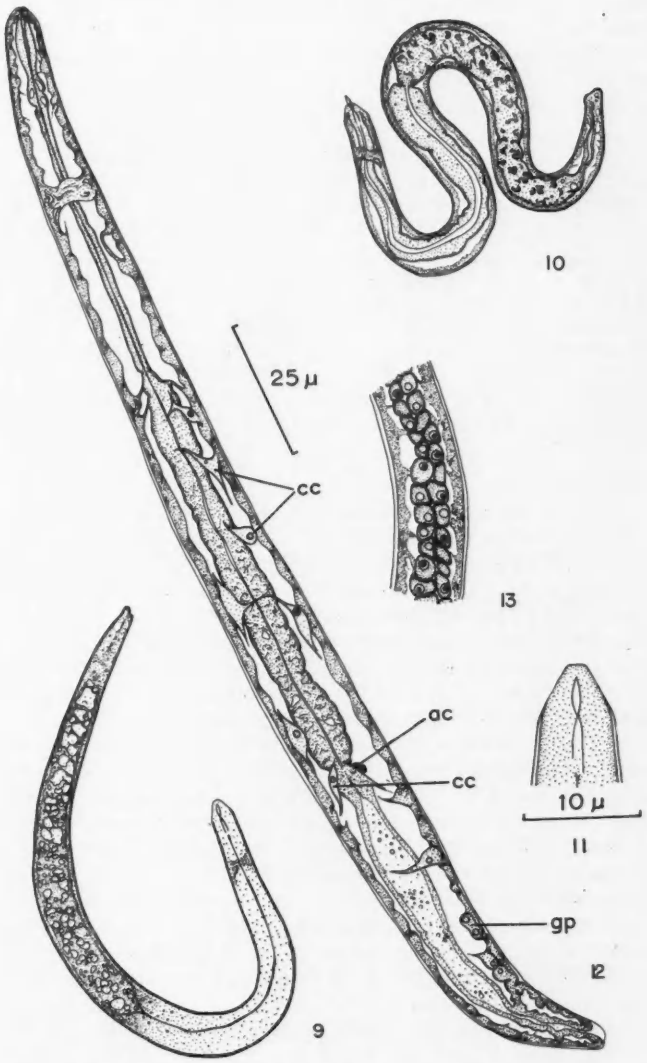


FIG. 9. Early first-stage larva squeezed from egg.
FIG. 10. Late first-stage larva from liver of a mouse 2 days after infection.
FIG. 11. Anterior end of first-stage larva.
FIG. 12. Second-stage larva from liver of a mouse 4 days after infection. *ac* = ventral cell; *cc* = coelomocyte; *gp* = genital primordium.
FIG. 13. Detail of stichosome of second-stage larva, stained with cotton blue.

First-stage larvae were first found in the liver after 52 hours, but were no more developed than those of the same age in the caecum. Molting first-stage larvae, in which the cuticle had loosened over the posterior end, were found in the liver after 3 and 4 days.

Measurements of all larvae are given in Table II.

TABLE II

Measurements (in microns) of larvae of *C. hepatica*

	First stage (9)*	Second stage (6)	Third stage (14)	Fourth stage (15)
Total length	140–190	160–220	270–670	1100–3600
Maximum diameter	7–11	10–14	16–33	23–38
Anterior oesophagus		42–69	63–98	150–370
Posterior oesophagus		63–79	90–283	320–1600
Nerve ring†		24–36	34–47	37–52
Length of spicule				220–330

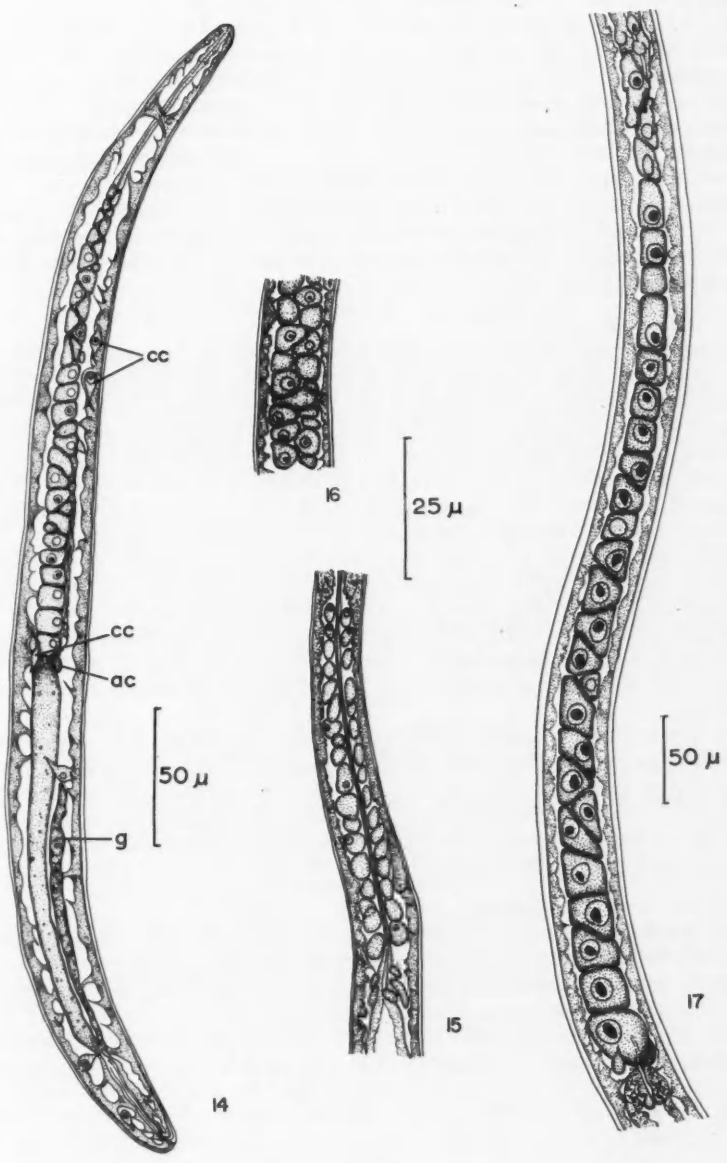
*Numbers in parentheses are numbers of larvae measured.
†Distance from anterior end.

The Second-stage Larva

Second-stage larvae were recovered from the liver of mice 3 to 7 days after feeding. The first-stage cuticle remained as a sheath throughout the second stage (Fig. 12). Its shape over the posterior end was characteristic since the cuticle did not extend into the rectum. Considerable differentiation took place during the second stage, but there was little increase in the length of the body (Table II). Most growth occurred in the oesophageal region. The second-stage larva had a clearly differentiated, cellular oesophagus and intestine, a small collar-like elevation around the oral opening, and lacked a stylet. The oesophagus was divided into a narrow, muscular part and a posterior stichosome. The structure of the latter will be considered in a separate section. The anterior oesophagus possessed a marked swelling immediately behind the buccal cavity followed by four large nuclei. A similar swelling, although less well marked, was situated anterior to the stichosome. These structures became progressively less distinct in subsequent larval stages. The short intestine occupied about one-fifth the total body length. The ventral, binucleate, genital primordium, situated midway between the oesophageal–intestinal juncture and the posterior extremity, developed no farther during this stage. The pseudocoelom contained many coelomocytes. A single compact cell lacking the extensions characteristic of coelomocytes occurred ventral to the oesophageal–intestinal juncture.

No second-stage larvae were found in the process of molting.

FIG. 14. Third-stage larva from liver of a mouse 7 days after infection. *ac* = ventral cell; *cc* = coelomocyte; *g* = gonad.
FIG. 15. Stichosome of molting first-stage larva from liver of a mouse 3 days after infection.
FIG. 16. Detail of stichosome of third-stage larva, stained with cotton blue.
FIG. 17. Stichosome of fourth-stage larva from liver of a mouse 9 days after infection.


cc
cc
ac
g
50 μ
14
16
25 μ
15
50 μ
17

The Third-stage Larva

Third-stage larvae were first recovered from the liver of mice 5 days after infection. The cuticular sheath formed by the second-stage cuticle extended inward to line the rectum of the third-stage larva. The collar-like elevation surrounding the oral opening of the second-stage larva was flatter and wider in the third stage, resembling more closely the structure of the adult. The genital primordium extended both anteriorly and posteriorly, but sexes could not be distinguished. The intestine had increased in length to occupy one-third the length of the body. The oesophageal–intestinal valve was more clearly defined. Most growth and differentiation occurred in the stichosome and gonad. The lateral bacillary bands were distinguished in this stage for the first time.

One larva, apparently molting to enter the fourth stage, was found 7 days after infection (Fig. 14).

The Fourth-stage Larva

Fourth-stage larvae were first found in the liver of mice on the ninth day after infection. Both male and female larvae were ensheathed in the third-stage cuticle. During the fourth stage the body length increased greatly (Table II), and the reproductive organs developed. Both the oesophagus and intestine increased greatly in length. The spicule, when first distinguished 12 days after infection, was solid and measured 215 μ in length. The spicule in two 14-day-old larvae measured 277 and 332 μ in length. After 16 days the spicule had increased in length to nearly the adult size and appeared tubular.

Males were considered adult when the ensheathing cuticle was lost after about 18 days. Similarly, females were considered adult after about 20 days. Egg-bearing females were found 21 days after infection. Female worms outlived male worms, the latter all having died in mice infected 40 days previously while living female worms were found as long as 59 days after mice were infected. No living female worms were found in mice examined 70 days after infection.

The larval development as outlined above for *C. hepatica* follows the pattern of molts described by Wehr (18) for *C. obsignata* (called *C. columbae* by Wehr). Wehr states, however, that in the late first-stage larva of *C. obsignata* the bacillary bands ". . . are feebly developed and extend the full length of the body . . .". In the present study, the bacillary bands were not seen in *C. hepatica* until the late third-stage larva.

Development of the Stichosome

The development of the stichosome was studied in both living and fixed larvae. Little cellular structure was distinguished in living larvae up to 9 days old, although the stichosome was quite apparent in larvae of the same age stained in cotton blue and cleared in glycerin.

Cells were first distinguished in the posterior part of the oesophageal region in living, molting, first-stage larvae (Fig. 15). At this time a loosely arranged, double row of 20 to 30 cells, the stichocytes, was distinguished in several living larvae. The cells were nearly spherical with relatively small nuclei. During the second stage the stichocytes enlarged and formed a more regular, closely packed, double column of cells (Fig. 13). During the third stage the

stichocytes became interdigitated and wedge-shaped (Fig. 16). By this time the nuclei were considerably enlarged and a single nucleolus in each could be seen. Development progressed more rapidly near the posterior end of the stichosome. Thus, in the molting third stage studied, the anterior stichocytes were wedge-shaped and interdigitated, while the posterior stichocytes were distinctly cuboid and arranged in a single row. In a 9-day-old larva (fourth stage) the stichocytes formed a single column, were considerably elongate, and the nuclei and nucleoli were considerably larger (Fig. 17). The oesophagus was not incorporated into the stichosome but lay lateroventrally to it. The greatest increase in the length of the oesophagus was achieved during the fourth stage by the elongation of the stichocytes. After 14 days the stichocytes resembled those of the adult. The nuclei in the adult were large and oval to pyriform. The nucleolus appeared to fragment into several irregularly shaped refractory bodies. It was not determined when the oesophagus was enclosed by the stichosome.

Basically, this development of the stichosome follows that outlined by Wu (19) for *Trichinella spiralis*. There is, however, no increase in number of stichocytes in the adult over the number first distinguished in molting first-stage larvae, and no evidence of "small cells" or of spiraling of stichocytes as reported by her for *T. spiralis*.

Acknowledgments

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MARINE NEMATODES FROM THE COAST LINE OF THE GULF OF MEXICO[1]

B. E. HOPPER

Abstract

A collection of marine nematodes from two locations along the coast line of the Gulf of Mexico is herein reported. Of the 14 species included, 9 species (*Lauratonema reniamphidum, Enoplolaimus punctatus, Oncholaimoides elongatum, Halichoanolaimus macrospiculatus, Desmodora dimorpha, Metachromadora* (*Neonyx*) *pseudocampycoma, M.* (*Bradylaimus*) *setosa, Neochromadora alatocorpus,* and *Prochromadora asupplementa*) are described as new. In addition, *Microlaimus dimorphus* Chitwood of Wieser, 1956 is renamed *M. wieseri* n. nom., and *Parachromadora* Timm, 1952 becomes *Timmia* n. nom., with new combinations *T. parva* (Timm, 1952) and *T. bipapillata* (Chitwood, 1951).

On several occasions during the years 1957 and 1958 the writer was afforded an opportunity to examine marine nematodes from the Alabama and Florida coasts of the Gulf of Mexico. The present paper includes only a small portion of the total number of species collected and is intended to serve as a basis for future articles by the author. Fourteen species are described, of which nine are new to science. In addition, new names are proposed for older species where it has been considered necessary.

Habitat Descriptions

With the single exception of *Anoplostoma viviparum*, which was collected from barnacles and mussels in the vicinity of Panama City, Florida, all species herein described came from Gulf Shores, Alabama. In general, the Gulf Shores specimens were collected from either exposed or sheltered sandy habitats. Two species came from a sheltered habitat with a muddy bottom.

Habitat 1.—Camp Helen, Panama City, Florida. Sample collected April 1958 by the author and E. J. Cairns. Specimens washed from barnacles and mussels attached to vegetation. Habitat sheltered. Salinity 27°/oo.

Habitat 2.—Gulf Shores, Alabama. Samples collected February 1957 and September 1958 by the author, and May 1958 by N. Sansing. Specimens washed from beach sand collected beyond low tide mark. Habitat exposed. Salinity 26.9°/oo.

Habitat 3.—Gulf Shores, Alabama. Samples collected February 1957 and September 1958 by the author. Specimens washed from sand collected in tidal pond. Habitat sheltered. Salinity 15.7°/oo.

Habitat 4.—Gulf Shores, Alabama. Sample collected by the author February 1957. Specimens washed from mud and silt collected in tidal pond. Habitat sheltered. Salinity 18°/oo.

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Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

List of Species and Name Changes

Family Lauratonematidae
Lauratonema reniamphidum n. sp.
Family Enoplidae
Enoplolaimus punctatus n. sp.
Family Ironidae
Dolicholaimus benepapillosus (Schulz, 1934) W. Schneider, 1939
Family Oncholaimidae
Anoplostoma viviparum (Bastian, 1865) Bütschli, 1874
Oncholaimoides elongatum n. sp.
Family Cyatholaimidae
Halichoanolaimus macrospiculatus n. sp.
Family Desmodoridae
Subfamily Desmodorinae
Desmodora dimorpha n. sp.
Subfamily Richsteriinae
Metachromadora (*Neonyx*) *pseudocampycoma* n. sp.
Metachromadora (*Bradylaimus*) *onyxoides* Chitwood, 1936
Metachromadora (*Bradylaimus*) *setosa* n. sp.
Family Microlaimidae
Microlaimus dimorphus Chitwood, 1936
Microlaimus wieseri n. nom.
syn. *M. dimorphus* Chitwood of Wieser, 1956
Family Chromadoridae
Timmia n. nom.
syn. *Parachromadora* Timm, 1952
nec *Parachromadora* Micoletzky, 1914 (subgenus of *Chromadora*)
nec *Parachromadora* Schulz, 1939
Timmia parva (Timm, 1952) n. comb.
syn. *Parachromadora parva* Timm, 1952
Timmia bipapillata (Chitwood, 1951) n. comb.
syn. *Prochromadorella bipapillata* Chitwood, 1951
Parachromadora bipapillata (Chitwood, 1951) Timm, 1952
Neochromadora alatocorpa n. sp.
Prochromadora asupplementa n. sp.

General Remarks

All observations were made on preserved specimens mounted in glycerine on Cobb slides. The spicular length recorded herein is the absolute spicular length and not the cord of the spicular arc. The types of all new species described are in the possession of the author.

Description of Species

LAURATONEMATIDAE Gerlach, 1953
Lauratonema Gerlach, 1953
Lauratonema reniamphidum n. sp.
(Figs. 1–3)

Male (*n*, 3).—Length 2.04 (1.97–2.1) mm; *a*, 68.1 (65.7–70); *b*, 6.5 (6.0–7.1); *c*, 12.1 (10.7–13.3).

Female.—Unknown.

Body long and slender, bearing fine striations. Head diameter 9.5 μ increasing to 13.5 μ at level of cephalic setae, 28 μ at base of neck, and 30 μ at midbody. Anal body diameter 29 μ. Head armed with six conical labial papillae and 10 cephalic setae 10.5 and 9.5 μ long. Amphids externally reniform, located just posterior to lateral cephalic setae, about one-fourth corresponding diameter in width.

Stoma spacious, 5 μ wide by 7 μ long, heavily sclerotized. Esophagus cylindrical, 9.5 μ wide at anterior, 10.5 μ at posterior extremity. Maximum esophageal diameter 13.5 μ, attained just in front of posterior end. Nerve ring encircling esophagus at 54% of its length. Cardia bluntly conoid, 9.5 μ wide and 10 μ long. Excretory pore 112 μ from anterior end.

Tail five anal body diameters long, curved ventrally, bearing six to eight pairs of subventral caudal and two terminal setae. Spicules bladelike, 20 μ long. The presence of a small platelike gubernaculum is questionable.

Differential diagnosis.—Lauratonema reniamphidum n. sp. is distinguished from all the known species except *L. mentulatum* Wieser (27) by having a spacious stoma, 1.6 times as long as wide. From *L. mentulatum* it differs in having shorter cephalic setae of equal length, smaller spicules, and reniform amphids situated posterior to the lateral cephalic setae.

Holotype.—Male on slide M30.

Paratypes.—Two males and two juveniles on slides M2 and M30A.

Occurrence.—Habitat 2.

Key to Species of Lauratonema Gerlach, 1953

1. Stoma spacious, 1.6 times longer than wide 2
   Stoma small, length equal to, or less than, width 3
2. Amphids anterior to cephalic setae; cephalic setae 19 and 14 μ long; spicules 26 μ long ..... *mentulatum* Wieser, 1959
   Amphids posterior to cephalic setae; cephalic setae 10.5 and 9.5 μ long; spicules 20 μ long ..... *reniamphidum* n. sp.
3. Cephalic setae of equal length 4
   Cephalic setae of unequal length 5
4. Cephalic setae short, equal to one-half corresponding diameter ..... *originale* Gerlach, 1956
   Cephalic setae longer, equal to corresponding diameter ..... *adriaticum* Gerlach, 1953
5. Male tail with three to four ventral papillae ..... *pugiunculus* Wieser, 1959
   Male tail without ventral papillae 6
6. Well developed gubernaculum present ..... *spiculifer* Gerlach, 1959
   Gubernaculum absent 7
7. Body form slender, *a* more than 65; length of long cephalic setae 8.5 μ ..... *hospitum* Gerlach, 1954
   Body form plumper, *a* less than 50; length of long cephalic setae 11 to 15 μ ..... *reductum* Gerlach, 1953

Family Enoplidae Baird, 1853
Subfamily Enoplinae [(Baird, 1853) Filipjev, 1918] Micoletzky, 1922
Enoplolaimus de Man, 1893
Enoplolaimus punctatus n. sp.
(Figs. 4–6)

Female (*n*, 4).—Length 2.44 (2.1–2.85) mm; *a*, 40.9 (37.8–45.6); *b*, 4.1 (3.8–4.3); *c*, 15.3 (13.1–16.7); *V*, 63.9 (59.7–70.9) %.

Male (*n*, 3).—Length 2.67 (2.66–2.7) mm; *a*, 53.4 (53.2–54); *b*, 3.9; *c*, 17.6 (15.9–19.7).

Body long and slender, female 61 μ, male 50 μ wide. Cuticle finely striated, annules bearing fine punctations. Cervical setae 10 μ long, somatic 8 μ long.

Head 30 μ wide, with finely striated, very thin, high, outwardly curled lips. Head anteriorly armed with six 12.5 μ long labial setae and two cirri (cephalic organs, cf. Wieser, 1953, page 59). Ten cephalic setae at base of 17 μ high cephalic capsule. Lateral cephalic setae 75–77 μ, the longer submedian, 67–70 μ, and the shorter submedian, 26–29 μ long. Distal extremities of long cephalic setae blunt with apparently open ends.

Stoma containing three 21 μ long mandibles with teeth 12 μ long. Esophagus cylindroid 580 to 690 μ long. Neck base diameter 47 μ wide.

Anal body diameter, 38 μ wide. Male tail 3.8, female 4.1 anal body diameters long. Spicules 30 μ long, gubernaculum one-half spicular length. Sclerotized supplementary organs lacking. (In ventral view a pore is recognizable a short distance preanal.)

Differential diagnosis.—*Enoplolaimus punctatus* n. sp. is related to *E. propinquus*, *derjugini*, *zosterae*, *glabra*, and *attenuatus* (see Wieser, 1953, table on p. 74). It differs from these species in possessing a punctate cuticle, by the absence of a sclerotized preanal supplementary organ, and by the spicules being shorter than the anal body diameter.

Holotype.—Male on slide M16.

Allotype.—Female on slide M15.

Paratypes.—Eleven males and two females of slides M14, M15, and M16.

Occurrence.—Habitat 2.

Family IRONIDAE de Man, 1876

Subfamily IRONINAE Micoletzky, 1922

Dolicholaimus de Man, 1888

Dolicholaimus benepapillosus (Schulz, 1934) W. Schneider, 1939

Syn. *Syringolaimus benepapillosus* Schulz, 1934

(Figs. 7–9)

Female.—Length 2.32 mm; *a*, 46.7; *b*, 9.4; *c*, 30.9; *V*, 57.6%.

Male No. 1.—Length 1.87 mm; *a*, 35.7; *b*, 6.9; *c*, 28.8; spicules 40 μ.

Male No. 2.—Length 2.00 mm; *a*, 44.6; *b*, 7.4; *c*, 30.0; spicules 43 μ.

Body relatively cylindrical, 50 μ wide, with thick, naked cuticle.

Head, at greatest diameter, 30 μ wide, set off by distinct groove, armed with 6 small labial and 10 large cephalic papillae. Amphids pocket-shaped, the apertures slitlike, occupying one-third the corresponding diameter. Stoma with rows of minute denticles. Teeth and apophyses of typical structure, together measuring 55 to 60 μ in length. Pharyngeal musculature 60 μ long by 20 μ wide, well separated from esophageal musculature. Esophagus uniformly conoid, 200 μ long, 18 μ wide at opposite end. Cardia bluntly conoid, well developed. Neck base diameter, 45 to 50 μ.

Anal body diameter 33 to 35 μ. Tail bluntly conical, in male 65 μ, in female 75 μ long. Structure of spicules and gubernaculum as illustrated (Fig. 9). Cuticle on ventral side of male body thickened 500 to 530 μ preanal, without supplements.

Remarks.—*D. benepapillosus* from the Gulf of Mexico differs rather noticeably from that described from Puget Sound by Wieser (27). While Wieser described and figured the head of his specimens as being conical, the cephalic contour of the Gulf of Mexico specimens is significantly more flattened with the cephalic papillae more prominent. The head contour of the Gulf of Mexico specimens corresponds extremely well with the illustration given by Schulz (21) in the original description of this species.

In addition, the Gulf of Mexico specimens differ from those of Puget Sound in having narrower bodies, shorter spicules, a heavier gubernaculum and by the absence of supplements.

Specimens.—Two males, one female, and three juveniles on slides M17 and M18.

Occurrence.—Habitat 3.

Family ONCHOLAIMIDAE (Filipjev, 1918) Baylis and Daubney, 1926
Subfamily ONCHOLAIMINAE [Filipjev, 1918] Micoletzky, 1922
Anoplostoma Bütschli, 1874
Anoplostoma viviparum (Bastian, 1865) Bütschli, 1874
Syn. *Symplocostoma vivipara* Bastian, 1865
Oncholaimellus heterurus Cobb, 1914

Female.—Length 1.2 mm; *a*, 32; *b*, 5.6; *c*, 7.2; *V*, 47.5%.
Male.—Length 1.2 mm; *a*, 34.3; *b*, 5.1; *c*, 8.0.

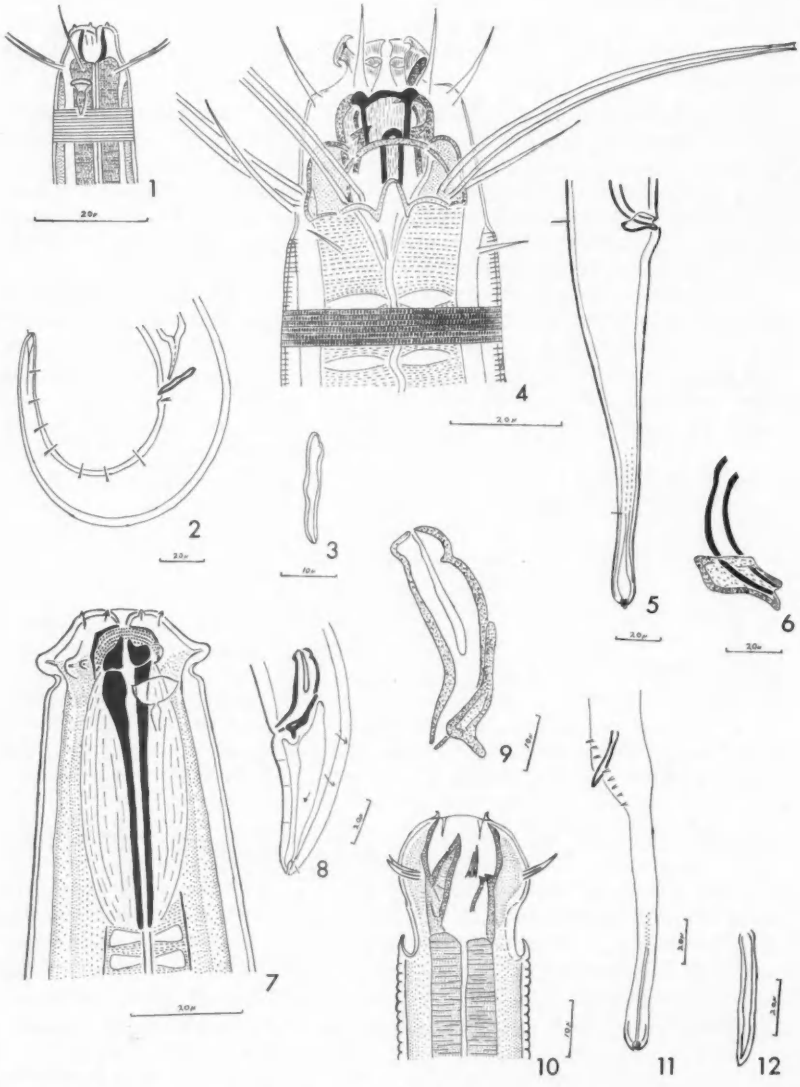
Cuticle smooth, without lateral alae. Sclerotized stoma 11 μ deep. Cephalic setae 10, the lateral and longer submedian 10 μ long, the four shorter submedian two-thirds the length of the longer. Esophagus 235 μ long, nerve ring at 53–54%. Female tail 8.5, male tail 6.2 anal body diameters long. Spicules 51.5 μ long.

Remarks.—The two adult specimens observed in this study were similar to those described by Timm (23) from Chesapeake Bay, Maryland. The single noticeable difference between the Gulf of Mexico specimens and those from Chesapeake Bay is found in the length of the spicules, being 51.5 and 40 μ long, respectively. However, this larger figure is in agreement with the figure recorded for Europenan specimens and agrees with the 50 to 56 μ spicular length of specimens recorded by Gerlach, 1957, from Brazilian waters.

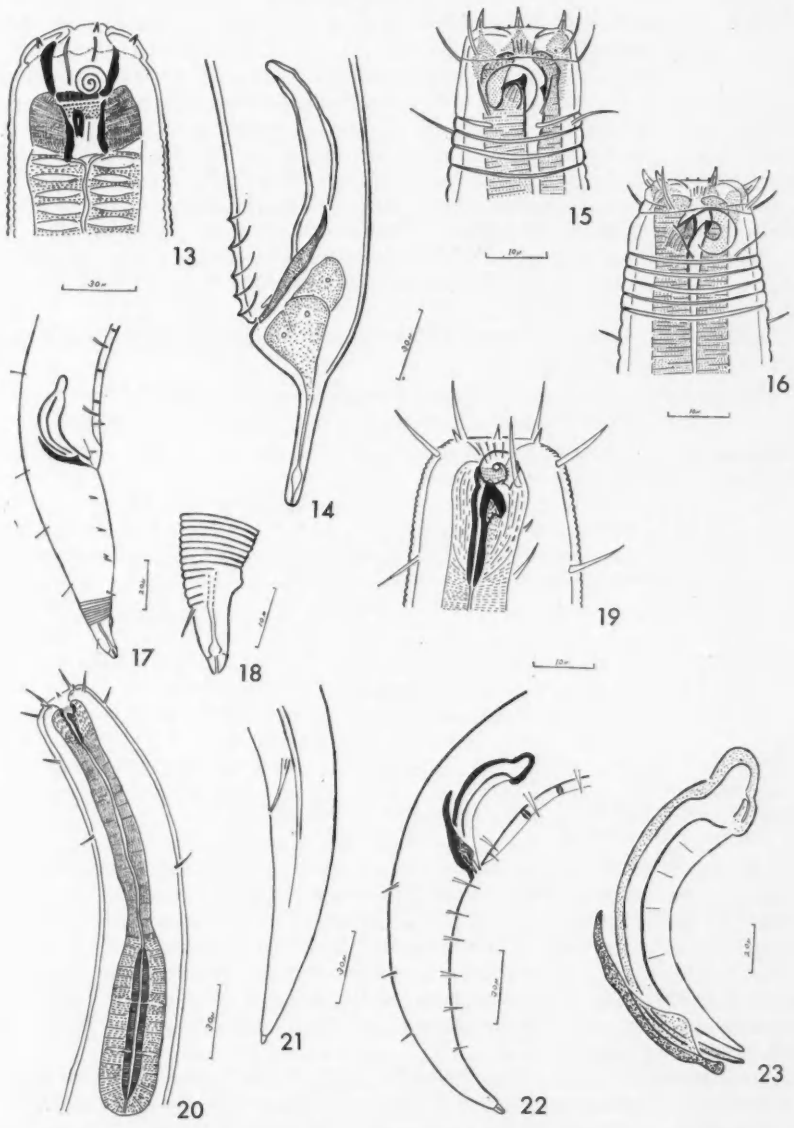
Specimens.—One male, one female, and one juvenile on slide M1.
Occurrence.—Habitat 1.

Oncholaimoides Chitwood, 1937
Oncholaimoides elongatum n. sp.
(Figs. 10–12)

Male.—Length 3.9 mm; *a*, 97.5; *b*, 7.1; *c*, 31.4.
Female.—Unknown.



FIGS. 1–3. *Lauratonema reniamphidum* n. sp. 1. Head of male. 2. Male tail. 3. Spicules.
FIGS. 4–6. *Enoplolaimus punctatus* n. sp. 4. Head of female. 5. Male tail. 6. Spicules and gubernaculum.
FIGS. 7–9. *Dolicholaimus benepapillosus* (Schulz). 7. Head of female. 8. Male tail. 9. Spicules and gubernaculum.
FIGS. 10–12. *Oncholaimoides elongatum* n. sp. 10. Head of male. 11. Male tail. 12. Spicules.



FIGS. 13–14. *Halichoanolaimus macrospiculatus* n. sp. 13. Head of male. 14. Male tail.
FIGS. 15–18. *Desmodora dimorpha* n. sp. 15. Head of male. 16. Head of female. 17. Male tail. 18. Posterior end of male tail showing ventral swelling anterior to terminus.
FIGS. 19–23. *Metachromadora* (*Neonyx*) *pseudocampycoma* n. sp. 19. Head of female. 20. Esophageal region of female. 21. Female tail. 22. Male tail. 23. Spicules and gubernaculum.

Body practically cylindrical from head to anal region; diameter at head 25 μ, at neck base 35 μ, at mid-region 40 μ, and at anus 25 μ. Diameter rapidly decreasing to 11 μ behind anus. Tail 125 μ long, the posterior 105 μ cylindrical. Terminus slightly clavate with spinneret. Cuticle coarsely annulated except anterior to base of amphids, bearing a few 3 μ long setae scattered on neck. Annules 1.7, 1, and 1.3 μ wide on neck, mid-body, and tail respectively. Each annule bearing delicate longitudinal markings. Head with six labial papillae and 10 cephalic setae 7 and 6 μ long. Stoma 55 μ deep, bearing a large acute dorsal tooth and two small subventrals.

Esophagus 410 μ long and 12 μ wide at anterior extremity gradually widening to 22 μ at its base. Nerve ring encircling esophagus at about 50%. Cardia bluntly conoid.

Spicules 24 μ long. Circumanal setae present. Gubernaculum and preanal supplements not observed.

Differential diagnosis.—*Oncholaimoides elongatum* n. sp. differs from the two described species in the greater body length, amphid size, and *a* value.

Holotype.—Male on slide M31.
Occurrence.—Habitat 2.

KEY TO SPECIES OF ONCHOLAIMOIDES CHITWOOD, 1937

1. Cuticle very coarsely striated; longitudinal markings pronounced . . *rugosum* Chitwood, 1937
   Cuticle finely striated; longitudinal markings delicate 2
2. Length 3.9 mm; *a*, 97.5 . . . . . *elongatum* n. sp.
   Length 2.1–2.7 mm; *a*, 29–37 . . . . . *striatum* Chitwood, 1937

Family CYANTHOLAIMIDAE De Coninck and Schuurmans Stekhoven, 1933
Subfamily CHOANOLAIMINAE Filipjev, 1934
Halichoanolaimus de Man, 1888
Halichoanolaimus macrospiculatus n. sp.
(Figs. 13, 14)

Male (*n*, 1).—Length 3.66 mm; *a*, 48.7; *b*, 7.3; *c*, 48.7.
Female.—Unknown.

Body long and cylindrical, 75 μ wide. Cuticle striated, the annules bearing punctations which are larger and more widely spaced laterally. Head 55 μ wide, armed with six very small cephalic setae. Amphid spiral of 2.75 turns, 13 μ wide, 18 μ from anterior extremity. Body diameter at level of amphid 60 μ. Stoma of two parts, anterior chamber 30 μ long by 30 μ wide separated from the 23 μ long by 15 μ wide posterior chamber by a circle of cuticular rods followed by several rows of denticles. Esophagus 500 μ long. Neck base diameter, 75 μ wide.

Anal body diameter, 45 μ. Tail 75 μ long of which the posterior 58 μ is cylindroid, 10 μ wide at start and 7 μ at spinneret. Spicules slightly arcuate, 115 μ long, maximum width 17.5 μ, distally acute. Gubernaculum approximately one-half as long as spicules. Five setose supplementary organs extending 45 μ preanal (one anal body diameter). Spicules extending 105 μ preanal.

Differential diagnosis.—*Halichoanolaimus macrospiculatus* n. sp. most closely resembles *H. robustus* (Bastian, 1865) de Man, 1888 but differs from it

in the longer spicules, fewer supplementary organs, and the nature of the tail. Following the grouping of Wieser (25), *H. macrospiculatus* belongs to the group of species having at least two-thirds of the tail cylindrical. It differs from all species in this group by the above-mentioned characters.

Holotype.—Male on slide M55.
Occurrence.—Habitat 4.

Family DESMODORIDAE (Filipjev, 1922) Micoletzky, 1924
Subfamily DESMODORINAE [Filipjev, 1922] Micoletzky, 1924
Desmodora de Man, 1889
Desmodora dimorpha n. sp.
(Figs. 15–18)

Female (*n*, one immature).—Length 1.87 mm; *a*, 56.8; *b*, 11.0; *c*, 20.4; V, 52%.

Male (*n*, 5).—Length 2.06 (1.95–2.16) mm; *a*, 68.8 (65–71.9); *b*, 10.9 (10.1–11.3); *c*, 25.3 (22.7–30.8).

Body long and slender, 30 μ wide. Cuticle heavily annulated, the annules 2.5 to 3 μ wide on neck region and 1.3 μ wide on remainder of body. Cuticle bearing six rows of setae, a dorsal, a ventral, and four sublateral. Setae of neck, numerous, 8 to 9 μ long, those posterior more widely spaced and 3 to 4 μ long.

Head 19 μ wide, armed with a circlet of six small labial papillae about oral opening, a primary circle of six stout, 5 μ long, cephalic setae at edge of head contour and a secondary circle of four 10 μ long cephalic setae about one-third down on the 12 μ long, non-perforated helmet. Amphids 9 μ wide, 43% of corresponding diameter; more or less a broad loop. Amphids displaying sexual dimorphism, in female of one wind, in male of a three-quarter wind. Two paramphidial setae, 10.5 μ long, situated posterior to and at sides of amphids (Figs. 15, 16).

Stomatal armature consisting of a large dorsal tooth opposed by a small ventral denticle. Stoma surrounded by esophageal tissue. Esophagus 190 (185–200) μ long, terminated by a small, tripartite, ovoid basal bulb, 43 μ long by 18 μ wide. Neck base diameter, 31 μ.

Anal body diameter, 29 μ. Tail 82.4 (70–92) μ long, about three anal body diameters. Male tail with subventral preanal and caudal setae. Cuticle ventrally thickened from anus to 350 μ preanal, with 13 to 14 supplements. Spicules 52.5 (50–55) μ long with 30 μ long gubernaculum. Male tail with ventral swelling anterior to terminus (Fig. 18).

Differential diagnosis.—*Desmodora dimorpha* n. sp. is most closely related to *D. inflexa* Wieser, 1956. It differs in that the males are shorter and thinner, have smaller amphids and head diameter, amphids of unclosed loop, paramphidial setae, and a somewhat longer tail with a subterminal ventral swelling. In addition, the spicules, as drawn by Wieser (26), are much narrower than those of *D. dimorpha*.

Holotype.—Male on slide M38.
Allotype.—Female on slide M38*a*.
Paratypes.—Four males and two juveniles on slides M38A and M38B.
Occurrence.—Habitat 2.

Subfamily RICHTERSIINAE [Kreis, 1927] Cobb, 1933
Metachromadora Filipjev, 1918
Metachromadora (*Neonyx*) *pseudocampycoma* n. sp.
(Figs. 19–23)

Male (*n*, 1).—Length 1.9 mm; *a*, 44.5; *b*, 8.7; *c*, 15.3.
Female (*n*, 2y).—Length 1.6 mm; *a*, 34.4; *b*, 8.9; *c*, 15.5; *V*, ?.

Body cylindrical, 43 μ wide. Cuticle finely striated with prominent lateral alae. Annules about 1 μ wide on neck. Cuticle bearing six rows of setae. Cervical setae 10 μ long. Four sublateral rows of somatic setae 13 to 14 μ long, the dorsal and ventral rows short, 3 μ long. Male tail with caudal and preanal pairs of subventral setae (Fig. 22).

Head diameter, 23 μ, armed with an inner circle of six 4 μ long cephalic setae and an outer circle of four, 14 μ long. A circle of six 11 μ long subcephalic setae follows. Amphids broadly spiral of one and a quarter winds, near contour of head. Stoma armed with denticles and large dorsal onchium. Pharyngeal musculature set off from esophageal tissue. Esophagus 220 μ long, with tripartite terminal bulb 80 μ long by 25 μ wide with heavily sclerotized lining. Neck base diameter, 40 μ.

Anal body diameter of male, 35 μ. Male tail 125 μ long. Spicules 80 μ long. Gubernaculum 52 μ long with distal lateral guiding flanges. Supplements 12, mere innervations in thickened ventral cuticle.

Differential diagnosis.—*Metachromadora* (*Neonyx*) *pseudocampycoma* n. sp. is very close to *M.* (*N.*) *campycoma* (Cobb, 1933). It differs in that the subcephalic and cervical setae are shorter than the longer of the cephalic setae, while in *M.* (*N.*) *campycoma* the opposite is true. *M.* (*N.*) *pseudocampycoma* lacks the specialization of the cuticle in the anal region into "cuticular papilla-like structures" reported for *M.* (*N.*) *campycoma*. In addition, the spicules are two anal body diameters long against "a little longer than anal body diameter" for *M.* (*N.*) *campycoma* and the anteriormost supplement innervation lies eight anal body diameters preanal while that of *M.* (*N.*) *campycoma* is recorded as being five anal body diameters preanal.

Holotype.—Male on slide M28.
Allotype.—Young female on slide M28A.
Paratype.—Young female on slide M28A.
Occurrence.—Habitat 2.

Metachromadora (*Bradylaimus*) *onyxoides* Chitwood, 1936
(Figs. 24–29)

Female (*n*, 3).—Length 1.87 (1.84–1.89) mm; *a*, 36.3 (33.4–37.9); *b*, 9.1 (8.8–9.5); *c*, 20.9 (20.4–21.5); *V*, 59.3 (57.9–61.2) %.
Male (*n*, 1).—Length 1.9 mm; *a*, 47.6; *b*, 10.6; *c*, 16.6.

Body cylindrical, female 50 μ, male 40 μ wide. Cuticle coarsely striated, annules on neck and body 1.4 μ wide, on tail 1.0 μ wide. Cuticle without lateral alae, bearing six longitudinal rows of somatic setae, a dorsal row 2 μ long, a ventral row 3.5 μ long, and four sublateral rows 18 to 19 μ long interspersed with 5 μ long setae. Somatic setae on neck 13 μ long. In vulvar region four

pairs of 11 μ long subventral setae appear. Subventral pairs of preanal and caudal setae of variable length occur on the male, being 4 to 5 μ long on caudal extremity, 15 μ long adanally, and 8 μ long preanally.

Head diameter 23 μ, armed with six short and four 11 μ long cephalic setae. Immediately behind cephalic setae occurs a single circle of eight 16 μ long setae which, in turn, is followed by a circle of four of 12 μ length. Amphids postlabial, broadly spiraled, 6 to 8 μ wide. Stoma without denticles, with large dorsal onchium, surrounded by distinct pharyngeal musculature. Esophagus in male 180 μ, in female 205 μ long, with tripartite terminal bulb two-fifths the total esophageal length long by 30 μ wide.

Vagina with bell-shaped sclerotizations (Fig. 27). Anal body diameter of male 40 μ, of female 30 μ. Tail of male 114 μ, of female 90 μ long. Spicules 60 μ long. Gubernaculum 40 μ long, distally with prominent lateral guiding flanges. Supplements 12, situated on medioventral elevation which extends 310 μ preanal.

Remarks.—*Metachromadora* (*Neonyx*) *onyxoides* from the Gulf of Mexico agrees extremely well with the original description and figures given by Chitwood (3). The Gulf of Mexico specimens vary from the type in their longer body measurements and in the male having 12, rather than 9 to 10, supplementary organs.

Specimens.—One male and four females on slide M27.
Occurrence.—Habitat 2.

Metachromadora (*Bradylaimus*) *setosa* n. sp.
(Figs. 30–33)

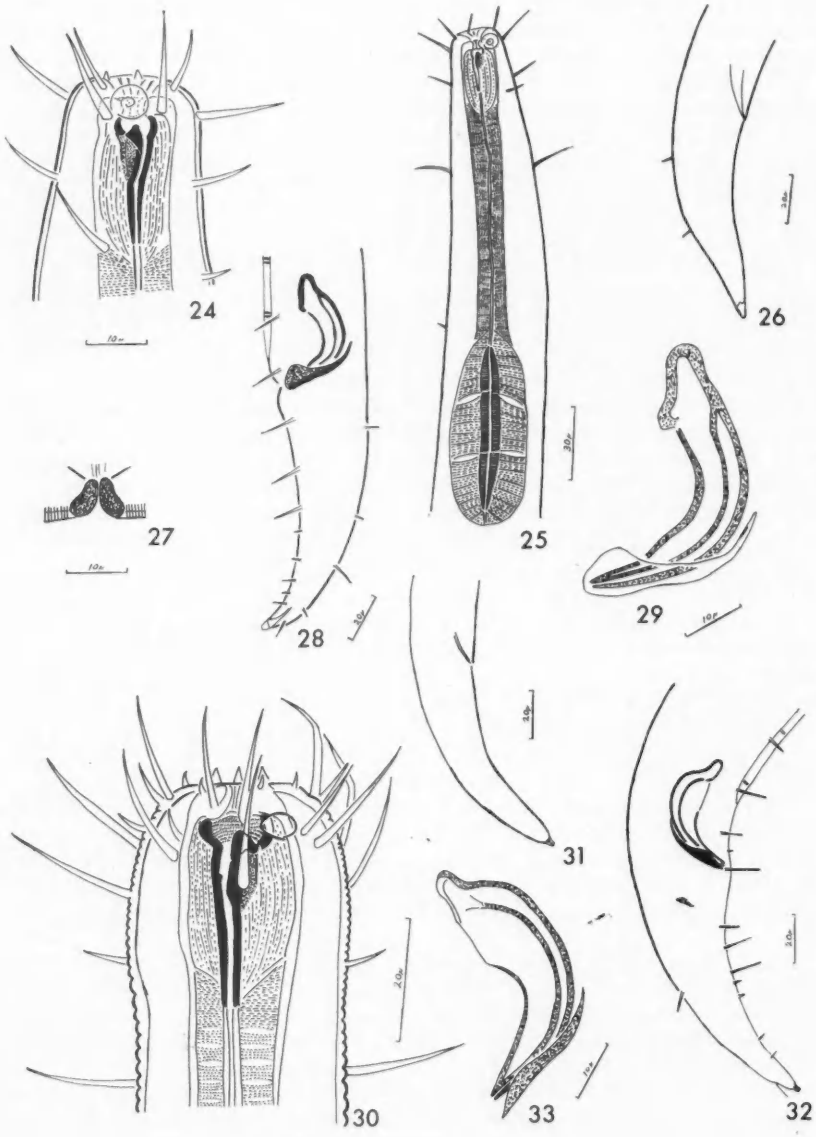
Female (*n*, 3).—Length 1.48 (1.3–1.57) mm; *a*, 26.9 (26–28.6); *b*, 6.4 (6.1–6.8); *c*, 16 (14.5–17.5); *V*, 55.9 (53.6–57.1) %.
Male (*n*, 2).—Length 1.89 (1.76–2.03) mm; *a*, 38.5 (32–45); *b*, 8 (7.4–8.6); *c*, 19.6 (17.6–21.6).

Body cylindrical, male 38 μ, female 55 μ wide. Cuticle coarsely striated, annules 3 μ wide on neck, 2 μ wide on remainder of body. Annules on head broken to form short rods of irregular length. Unbroken annules commencing 30 μ from head end. Cuticle without lateral alae, bearing six longitudinal rows of setae, a dorsal 5 μ long, a ventral 6 μ long, and four sublateral up to 20 μ long.

Head diameter 40 μ, bearing numerous circles of setae. The cephalic setae consist of 10 setae, 6 of which are 19 μ long and the remaining 4, 8 μ long. Anterior to the cephalic setae are two circles of six 3 μ long setae. Behind the cephalic setae are several circles of eight setae, one of 7 μ long, followed by circles of setae 22 μ long, 25 μ long, 8 μ long, and 23–25 μ long.

Stoma 15 μ wide anteriorly, provided with denticles and armed with large dorsal onchium. Pharyngeal musculature distinct from esophageal tissue. Esophagus 240 μ long with elongate tripartite terminal bulb, 120 μ long by 30 μ wide with heavily sclerotized lining. Neck base diameter, 42 μ.

Anal body diameter, 40 μ. Spicules 60 μ long. Gubernaculum 38 μ long. Medioventral cuticular elevation bearing 11 weak supplementary organs.



FIGS. 24–29. *Metachromadora* (*Bradylaimus*) *onyxoides* Chitwood. 24. Head of female. 25. Esophageal region of female. 25. Female tail. 27. Sclerotization of vulvar region. 28. Male tail. 29. Spicules and gubernaculum.

FIGS. 30–33. *Metachromadora* (*Bradylaimus*) *setosa* n. sp. 30. Head of male. 31. Female tail. 32. Male tail. 33. Spicules and gubernaculum.

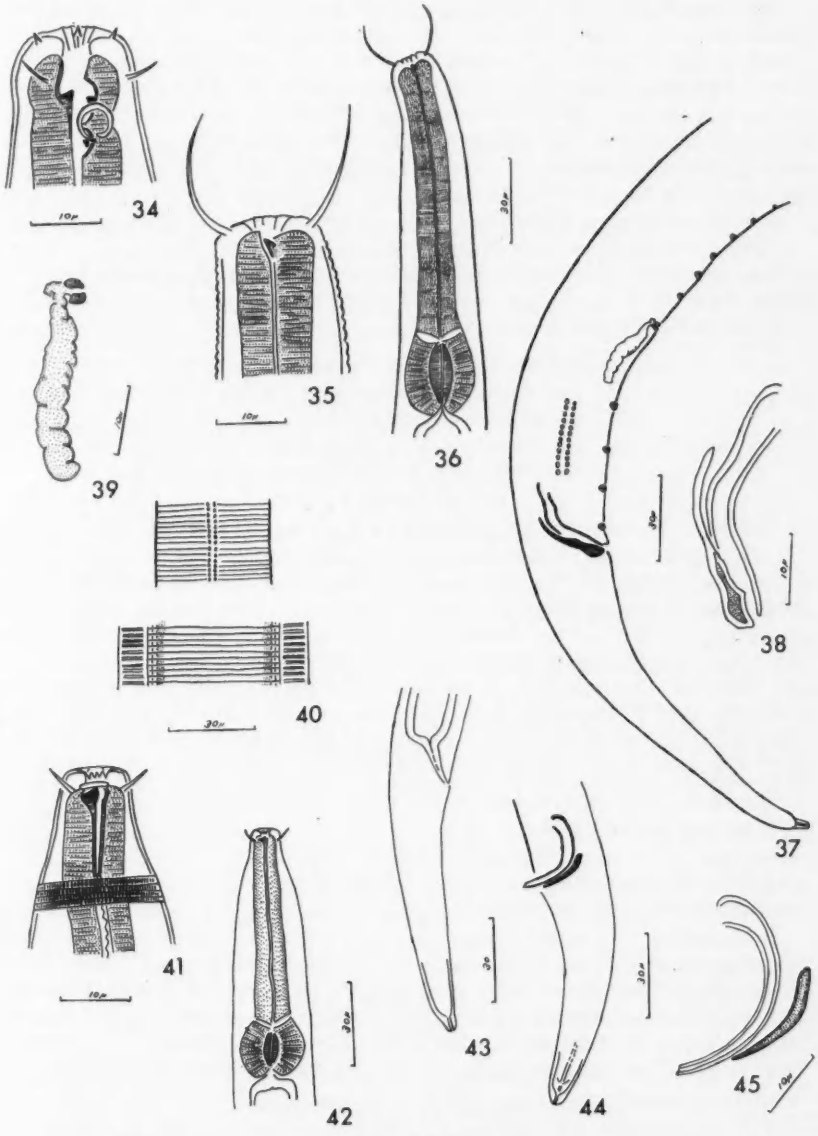


FIG. 34. *Microlaimus dimorphus* Chitwood. Head of female.

FIGS. 35–40. *Neochromadora alatocorpa* n. sp. 35. Head of female. 36. Esophageal region of female. 37. Male tail. 38. Spicules and gubernaculum. 39. Glandular modification of fifth preanal supplement. 40. Body widths in lateral view and dorsal–ventral view.

FIGS. 41–45. *Prochromadora asupplementa* n. sp. 41. Head of female. 42. Esophageal region of female. 43. Female tail. 44. Male tail. 45. Spicules and gubernaculum.

Differential diagnosis.—Following the scheme of Gerlach (10), *Metachromadora setosa* n. sp. belongs to the subgenus *Bradylaimus*. It differs from both species in this subgenus by the esophageal bulb being one-half the total length of the esophagus, whereas in *M.* (*B.*) [*onyxoides* it is two-fifths and in *M.* (*B.*)] *suecica* it is one-third the total length. In addition, it differs from both species in the pilosity of the cephalic region and in having a denticulate stoma. In the latter characteristics the species is very similar to *Sigmophora litoralis* (Schulz, 1939).

Holotype.—Male on slide M28C.

Allotype.—Female on slide M28B.

Paratypes.—One male, three females, and three juveniles on slides M28D, M28E, and M28F.

Occurrence.—Habitat 2.

Family MICROLAIMIDAE (Micoletzky, 1922) De Coninck and Schuurmans Stekhoven, 1933

Subfamily MICROLAIMINAE Micoletzky, 1922

Microlaimus de Man, 1880

Microlaimus dimorphus Chitwood, 1937

(Fig. 34)

Female (*n*, 1).—Length 1.2 mm; *a*, 18.5; *b*, 7.6; *c*, 10.4; *V*, 53.6%.

Body rather obese, 65 μ wide, tapering towards the extremities. Cuticle transversely striated, bearing scattered 3 μ long setae. Annules near head about 1.0 μ wide. Cephalic sensory organs consisting of an anterior circle of six setose papillae located on contour of head and a posterior circle of four 5 μ long cephalic setae. Amphids opposite stomatal region, 5.2 μ wide (corresponding diameter 19 μ). Stoma as illustrated (Fig. 34). Stomatal musculature distinctly set off. Excretory pore 26 μ posterior to cephalic extremity. Esophagus 157 μ long with terminal esophageal bulb 26 μ long by 29 μ wide. Anal body diameter 34 μ. Rectum 26 μ long. Uteri containing many spermatozoa.

Specimen.—One female on slide M3.

Occurrence.—Habitat 3.

Microlaimus wieseri nov. nom.

Syn. *M. dimorphus* Chitwood of Wieser, 1956

nec *M. dimorphus* Chitwood, 1937

In recording *Microlaimus dimorphus* from Chile, Wieser (26) described the excretory pore as being located near the esophageal bulb. The present study clearly shows that Chitwood (4) was correct in his observation of the excretory pore position. It therefore appears that Wieser had a species closely related to *M. dimorphus* of Chitwood (4), but differing from it in the location of the excretory pore. For this species the name *Microlaimus wieseri* is proposed.

Family CHROMADORIDAE Filipjev, 1917

Subfamily CHROMADORINAE [Filipjev, 1917] Micoletzky, 1922

Timmia nov. nom.

Syn. *Parachromadora* Timm, 1952

nec *Parachromadora* Micoletzky, 1914

(Subgenus of *Chromadora*)

nec *Parachromadora* Schulz, 1939

This genus has the characters of the genus *Parachromadora* Timm, 1952.

Timmia parva (Timm, 1952) nov. comb.

Syn. *Parachromadora parva* Timm, 1952

Female (*n*, 1).—Length 0.838 mm; *a*, 25.4; *b*, 7.6; *c*, 7.6; *V*, 46.3%.

Male (*n*, 1).—Length 0.750 mm; *a*, 27.8; *b*, 6.8; *c*, 10.

Female 33, male 27 μ wide. Cuticular ornamentation homogeneous, without lateral differentiation, lateral pores, cervical and somatic setae. Head 10 μ wide, armed with four 5 μ long cephalic setae. Amphid 4.2 μ wide, transversely oval. Esophageal bulb 23 μ long by 20 μ wide. Neck base diameter 25 μ in male, 28 μ in female. Anal body diameter, 20 μ.

Spicules 27 μ long. Gubernaculum 24 μ long. Male with prominent tubular curved supplement 3 μ preanal followed by two regular chromadoroid supplements 10 μ and 30 μ preanal (cf. Timm, 1952, page 21, figs. 38 and 39).

Specimens.—One male and one female on slide M35.

Occurrence.—Habitat 3.

Other Species

Timmia bipapillata (Chitwood, 1951) nov. comb.

Syn. *Prochromadorella bipapillata* Chitwood, 1951

Parachromadora bipapillata (Chitwood, 1951) Timm, 1952

Neochromadora Micoletzky, 1924

Neochromadora alatocorpa n. sp.

(Figs. 35–40)

Female (*n*, 2).—Length 0.975 and 1.05 mm; *a*, 21.7 and 26.2; *b*, 7.8 and 7.9; *c*, 8.0 and 8.1; *V*, 48.1 and 47.1%.

Male (*n*, 5).—Length 1.064 (0.975–1.13) mm; *a*, 26.9 (26.0–28.1); *b*, 8.52 (7.78–8.87); *c*, 8.23 (7.67–8.46).

Body relatively stout, 40 to 45 μ wide. Cuticle striated, ornamentation heterogeneous, laterally differentiated by 12 μ wide lateral alae. Cervical setae 9 μ long, somatic setae up to 20 μ long, arranged in two rows, one on either side of lateral alae.

Head 15 μ wide, armed with four 25 μ long cephalic setae. Amphids reniform, situated at level of cephalic setae. Stoma armed with prominent, triangular, hollow, dorsal tooth. Cylindrical esophagus terminated by basal bulb 30 μ long by 25 μ wide in female and 25 μ long by 19 μ wide in male. Neck base diameter 28 to 33 μ.

Anal body diameter 26 to 30 μ. Spicules 41.6 (37–45) μ long. Gubernaculum two-thirds length of spicules, with distal lateral guiding flanges (Fig. 38). Supplements 11, small, chromadoroid. Fifth preanal supplement modified into 27 μ long posteriorly extending glandular organ (Fig. 39).

Differential diagnosis.—*Neochromadora alatocorpa* n. sp. belongs to the group of species with conspicuous triangular teeth, complicated cuticular ornamentation and long cephalic setae (cf. Wieser (25, page 85)). It differs from all the members of the group by the long cephalic setae and the glandular modification of the fifth supplement.

Remarks.—The width of the nema given above as 40 to 45 μ has been derived from a dorsal–ventral measurement. Due to the width of the lateral alae a measurement in the lateral plane gives a width of 70 μ.

Holotype.—Male on slide M21.
Allotype.—Female on slide M22.
Paratypes.—Four males and two females on slides M21 and M22.
Occurrence.—Habitat 2.

Prochromadora Filipjev, 1922
Prochromadora asupplementa n. sp.
(Figs. 41–45)

Female (*n*, 1).—Length 0.657 mm; *a*, 20.2; *b*, 7.5; *c*, 7.3; *V*, 45.2%.
Male (*n*, 1).—Length 0.642 mm; *a*, 23.3; *b*, 6.9; *c*, 7.6.

Body relatively stout, 28 to 32 μ wide. Cuticle striated, annules 1.3 μ wide, bearing ovoid punctations which become rounded and wider spaced on tail, homogeneous, without lateral alae (no lateral differentiation). Short, 2 μ long, somatic setae arranged in a single row on either side of the underlying lateral cords.

Head 10 μ wide, armed with four 5 μ long cephalic setae. Narrow, transversely elongate amphid situated laterally at level opposite cephalic setae. Circle of four pairs of short submedian cervical papillae 25 μ behind head end.

Stoma armed with large dorsal tooth opposed by ventral cavity. Esophagus cylindrical to terminal bulb. Terminal bulb non-divided, 20 μ long by 24 μ wide. Neck base diameter 28 μ.

Anal body diameter 22 μ in female, 25 μ in male. Tail tapering gradually to rather stout spinneret-bearing terminus. Tail diameter at spinneret 7 to 8 μ. Spicules slender, 38.5 μ long, strongly arcuate, proximally somewhat cephalated. Gubernaculum 16 μ long, without apophysis or distal lateral guiding flanges. Supplementary organs lacking.

Female amphidelphic, ovaries reflexed. Egg 49 μ long by 29 μ wide.

Differential diagnosis.—*Prochromadora asupplementa* n. sp. differs from all the other species in the genus by the absence of supplementary organs.

Holotype.—Male on slide M13.
Allotype.—Female on slide M13.
Occurrence.—Habitat 2.

Acknowledgments

The author wishes to express his appreciation to Dr. B. G. Chitwood, formerly of the Kaiser Foundation Research Institute, Richmond, California, to Fr. R. W. Timm, Notre Dame College, Dacca, East Pakistan, and to Dr. Wolfgang Wieser, Zoologisches Institut der Universitat Wien, Wien, Austria, for reviewing the manuscript and offering their suggestions for its improvement. The author wishes to especially extend his appreciation to Dr. Wolfgang Wieser for the many hours spent with the author studying specimens at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, during the latter part of 1958. In addition, the author wishes to thank the Woods Hole Oceanographic Institution and Auburn University for making the study at Woods Hole financially possible.

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SPLENDIDOFILARIA WEHRI N.SP. WITH A REVISION OF SPLENDIDOFILARIA AND RELATED GENERA[1]

ROY C. ANDERSON

Abstract

Splendidofilaria wehri n. sp. from the heart of *Hylocichla ustulata swainsoni* is described and compared with closely related species. Species formerly allocated to *Splendidofilaria* and *Ornithofilaria* are reorganized on the basis of the structure of the oesophagus as follows: (*a*) *Splendidofilaria* Skrjabin, 1923 (=*Ornithofilaria* Gönnert, 1937; *Ularofilaria* Lubimov, 1946; *Lophortofilaria* Wehr and Herman, 1956); *pawlowskyi* Skrjabin, 1923, *gedoelsti* Travassos, 1925, *brevispiculum* Singh, 1949, *verrucosa* Oschmarin, 1950, *kashmirensis* Amir and Ali, 1960, *wehri* n.sp., *mavis* (Gönnert, 1937) n.comb., *papillocerca* (Lubimov, 1946), *rotundicephala* (Oschmarin, 1950) n.comb., *fallisensis* (Anderson, 1954) n.comb., *algonquinensis* (Anderson, 1955) n.comb., *californiensis* (Wehr and Herman, 1956) n.comb., *tuvensis* (Spassky and Sonin, 1957) n.comb., *böhmi* (Supperer, 1958) n.comb., *gretillati* Chabaud, Anderson and Brygoo, 1959. (*b*) *Chandlerella* Yorke and Maplestone, 1926 (=*Vagrifilaria* Augustine, 1937; *Parachandlerella* Caballero, 1948); *bosei* (Chandler, 1924), *travassosi* (Koroliowa, 1926) n.comb., *skrjabini* (Petrov and Tschertkowa, 1947) n.comb., *columbigallinae* (Augustine, 1937) n.comb., *stantchinskyi* Gilbert, 1932, *sinensis* Li, 1933, *lienalis* Orloff, 1947, *periarterialis* (Caballero, 1948) n.comb., *braziliensis* Yeh, 1957, *singhi* Ali, 1957.

Introduction

A new species of *Splendidofilaria* has been found in the heart muscle of an olive-backed thrush from Algonquin Park, Ontario, Canada. It is described herein and named in honor of Dr. Everett E. Wehr of the Animal Disease and Parasite Research Division, United States Department of Agriculture, Beltsville, Maryland.

In addition, an attempt is made to reclassify the numerous species that have been assigned to *Splendidofilaria* Skrjabin, 1923, *Ornithofilaria* Gönnert, 1937, and related genera.

Splendidofilaria wehri n.sp.

(Figs. 1–10)

Description

General.—Filarioidea, Onchocercidae, Splendidofilariinae, *Splendidofilaria* Skrjabin, 1923. Slender worms with slightly attenuated extremities. Buccal capsule absent, oral opening minute. Amphids minute, lying outside four more or less submedian papillae. Oesophagus long, narrow, exhibiting no evidence of glandular or muscular tissue, displaced ventrally at its anterior extremity. Intestine broad, full of refractory material (? erythrocytes). Caudal extremity in both sexes terminating in pair of delicate, fleshy, lateral protuberances. Minute phasmids some distance from caudal extremity. Cuticle thin, with delicate, regular, transverse striations, and carrying transversely elongated

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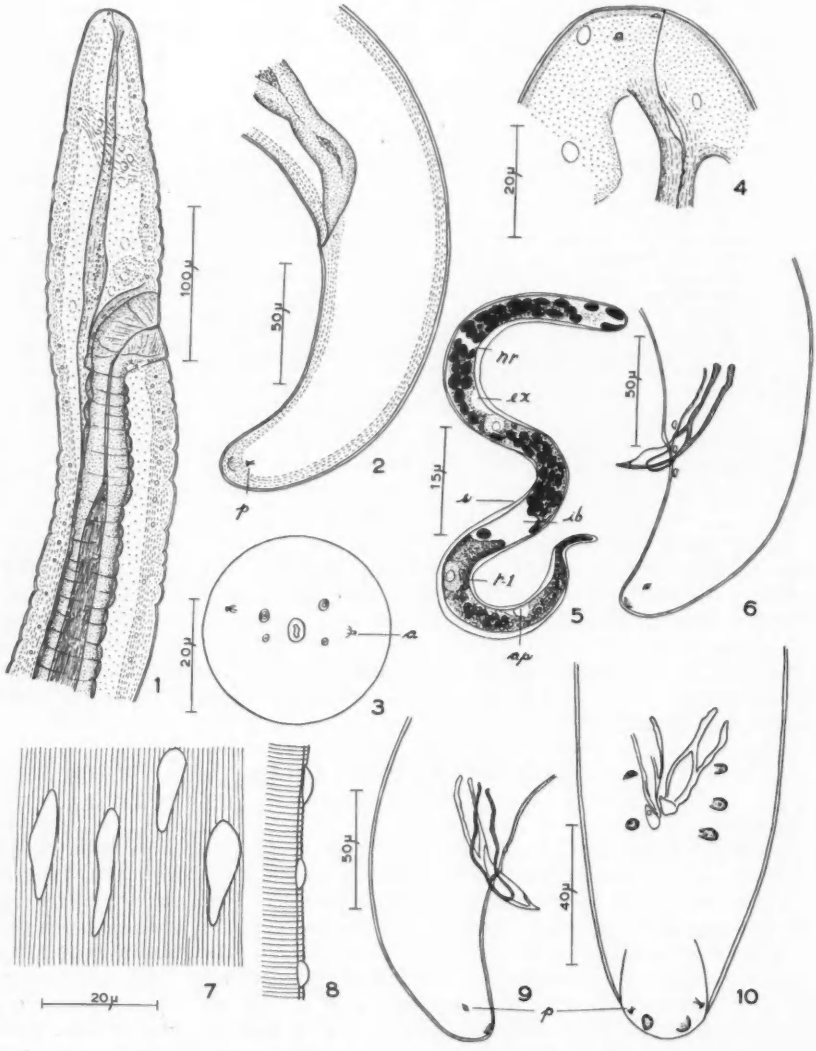

FIGS. 1–10. *Ornithofilaria wehri* n. sp.

FIG. 1. Anterior end female, lateral view. FIG. 2. Caudal end female, lateral view. FIG. 3. Anterior end female, *en face* view. FIG. 4. Anterior end female, lateral view. FIG. 5. Microfilariae (Giemsa). FIG. 6. Posterior end male, lateral view. FIG. 7. Cuticle of female showing bosses, surface view. FIG. 8. Cuticle of female showing bosses, side view. FIG. 9. Posterior end male, lateral view. FIG. 10. Posterior end male, ventral view.

ABBREVIATIONS: *a* = amphid; *ap* = anal pore; *ex* = excretory pore; *ib* = inner body; *nr* = nerve ring; *p* = phasmid; *r*-1 = first rectal cell; *s* = sheath.

bosses except at extremities. Excretory pore not discerned with certainty, and deirids apparently absent.

Male (holotype).—Length 9.4 mm. Maximum width 133 μ near middle of body. Oesophagus 290 μ in length. Nerve ring 71 μ from cephalic extremity. Anus 63 μ from rounded caudal extremity. Caudal papillae consisting of two on the right side and three on the left, all being in the region of anus. Spicules subequal, dissimilar morphologically; left 65 μ in length ending in point and clearly divided into two portions, right 51 μ in length, terminal end rounded and spatulate in form.

Female (three specimens, first figure allotype).—Length 19, 17, 21 mm. Maximum width 222, 166, 211 μ near middle of body. Oesophagus 466, 396, 366 μ in length. Nerve ring 69, 69, 76 μ from cephalic extremity. Vulva 187, 185, 211 μ from cephalic extremity, not opening on elevation. Vagina 480, 327, 317 μ in length, directed posteriorly for all its length and dividing into two broad uteri packed with microfilariae. Anus barely patent, 139, 106, 113 μ from caudal extremity.

Microfilaria (Giemsa, 10 specimens).—Length of microfilariae dissected from preserved female specimen about 125 μ, but on blood films 90–106 (av. = 99) μ. Maximum width 3–4 μ. Surrounded by delicate hyaline sheath. Cephalic space occupied by two deeply staining nuclei. Caudal end attenuated, ending in blunt point. Inner body represented by poorly demarcated clear space. Positions of fixed points expressed as percentages of total length as follows: nerve ring 22–26%; excretory pore 31–35%; excretory cell 34–38%; anterior border of inner body 52–59%; posterior border of inner body 57–64%; first rectal cell 65–73%; anus 79–85%.

Host.—Olive-backed thrush, *Hylocichla ustulata swainsoni* (Tschudi). Turdidae.

Location.—Imbedded in wall of right atrium; microfilariae in blood.

Locality.—Algonquin Park, Ontario, Canada.

Specimens.—United States National Museum Nos. 56809–56810.

Specific diagnosis.—*S. wehri* is close to four other members of the genus possessing cuticular bosses namely, *pawlowskyi*, *brevispiculum*, *verrucosa*, and *kashmirensis*. The descriptions of some of these species are not complete; the microfilaria of none of them has been described in detail. However, its short spicules will separate *wehri* from *kashmirensis* in which the right is 78–83 μ and the left 95–120 μ in length. Its well-developed caudal papillae situated near the anus distinguishes *wehri* from *verrucosa*, which has only two pairs of minute papillae on the tail. The microfilaria of *brevispiculum* is said to be only 82 μ in length and the oesophagus of this species is considerably longer (male 630–740 μ; female 873 μ). Similarly, the oesophagus in *pawlowskyi* is longer and there are four pairs of well-developed papillae distributed at equal distances along the tail. It is worth noting that all these species were from the heart or heart tissue except *kashmirensis*, which was found in the body cavity of its host.

Discussion

Chabaud and Choquet (1953) (11) wisely rejected the use of cuticular bosses as generic characters of filarioids close to *Splendidofilaria* and *Ornithofilaria* and provisionally separated the former from the latter group on the basis of

the length of the tail, which is usually long and digitiform in *Splendidofilaria* and short and rounded in the others. Since this early work a number of species have been described and it is possible now to reorganize these filarioids less arbitrarily. Chabaud and Choquet's contention that cuticular bosses are useless as generic characters is borne out by the morphological similarity of *wehri* n.sp. to *fallisensis*, *mavis*, *algonquinensis*, etc., which lack them. There are, however, differences in the oesophagi of these nematodes and by using these characters some remarkably homogeneous groups can be formed. A few species have a short, stout, club-shaped oesophagus (e.g. *bosei* (Chandler, 1924)). In some the oesophagus is long and prominently divided externally into two parts, e.g. *columbigallinae* (Augustine, 1937). Finally, in a large group, the oesophagus is narrow, undivided, imperfectly separated from the intestine and evidently devoid of glandular tissue. The latter type of oesophagus has been considered characteristic of *Ornithofilaria* but it is found in some species of *Splendidofilaria*, notably *pawlowskyi*. The form of the oesophagus in the developmental stages of *fallisensis* in simuliids is similar to that of the adult (5). The author thinks this character is of fundamental importance and must be used as a generic character. On the basis of the morphology of the oesophagus the following arrangement of the numerous species assigned to *Ornithofilaria*, *Splendidofilaria*, *Chandlerella*, *Vagrifilaria*, etc. is proposed.

SUBFAMILY SPLENDIDOFILARIINAE CHABAUD AND CHOQUET, 1953*

Diagnosis.—Onchocercidae. Vulva in oesophageal region. Caudal alae absent; caudal papillae reduced in size. Spicules subequal, different in morphology. Tail short or long, never terminal or subterminal. Parasites of birds, reptiles, and mammals.

Splendidofilaria SKRJABIN, 1923

Syn.—*Ornithofilaria* Gönnert, 1937; *Ularofilaria* Lubimov, 1946; *Lophortofilaria* Wehr and Herman, 1956.

Amended diagnosis.—Splendidofilariinae. Oesophagus long, narrow, undivided, apparently devoid of glandular tissue and imperfectly demarcated from intestine. Spicules subequal but different in morphology. Cuticle sometimes with bosses. Vulva anterior to end of oesophagus. Caudal papillae often much reduced, usually in rows on ventrolateral surface of caudal extremity. Two pairs of cephalic papillae and amphids present, often arranged asymmetrically about stoma. Tail usually terminating in a pair of fleshy protuberances (not phasmids). Microfilaria short. Parasites of the blood vascular system, subcutaneous tissues, and serous cavities of birds.

A. Cuticular Bosses Present

1. *S. pawlowskyi* Skrjabin, 1923 (type species). *Hosts*: *Otomela isabellina*, *O. phaenicuroides* (Laniidae). *Location*: cavity of heart. *Locality*: U.S.S.R. (22).
2. *S. gedoelsti* Travassos, 1925. *Host*: *Selenidera maculirostris* (Rhampha-

*It is necessary to suppress Ornithofilariinae Chabaud and Anderson, 1959 (9) incorporating its genera into Splendidofilariinae. Apart from this change no alteration in the keys to the Onchocercidae given by Chabaud and Anderson, 1959 is necessary. The status of *Skrjabinocta petrowi* Tschertkowa, 1946 (27) from *Streptopelia orientalis meena* remains in doubt.

stidae). *Location:* subcutaneous. *Locality*: Brazil (26).

3. *S. brevispiculum* Singh, 1949. *Hosts*: *Streptopelia decacocto* (Columbidae), *Halcyon smyrensis* (Halcyonidae). *Location*: cavity of heart. *Locality*: India (21).
4. *S. verrucosa* Oschmarin, 1950. *Host*: *Garrulus glandarius* (Coraciidae). *Location*: heart. *Locality*: U.S.S.R. (19).
5. S. *kashmirensis* Amir and Ali, 1960. *Host*: *Molpastes l. leucogenys* (Pycnonotidae). *Location*: body cavity. *Locality*: Kashmir (2).
6. *S. wehri* n.sp.

B. Cuticular Bosses Absent

7. *S. mavis* (Gönnert, 1937) n.comb. Syn. *Ornithofilaria mavis*. *Host*: *Turdus musicus* (Turdidae). *Location*: subcutaneous. *Locality*: Britain and Germany (16, 14).
8. *S. papillocerca* (Lubimov, 1946) Anderson and Chabaud, 1959. Syn. *Ularofilaria papillocerca*. *Host*: *Tetraogallus altaica* (Tetraonidae). *Location*: subcutaneous. *Locality*: U.S.S.R. (18, 6).
9. *S. rotundicephala* (Oschmarin, 1950) n.comb. Syn. *Ornithofilaria rotundicephala*. *Host*: *Garrulus glandarius* (Coraciidae). *Location*: orbit. *Locality*: U.S.S.R. (19).
10. *S. fallisensis* (Anderson, 1954) n.comb. Syn. *Ornithofilaria fallisensis*. *Host*: *Anas boschas domesticus* (Anatidae). *Location*: subcutaneous. *Locality*: Ontario, Canada (3).
11. *S. algonquinensis* (Anderson, 1955) n.comb. Syn. *Ornithofilaria algonquinensis*. *Host*: *Hirundo erythrogaster* (Hirundinidae). *Location*: pulmonary artery. *Locality*: Ontario, Canada (4).
12. *S. californiensis* (Wehr and Herman, 1956) n.comb. Syn. *Lophortofilaria californiensis*. *Host*: *Lophortyx californicus* (Tetraonidae). *Location*: heart ventricles. *Locality*: California, U.S.A. (28).
13. *S. tuvensis* (Spassky and Sonin, 1957) n.comb. Syn. *Ornithofilaria tuvensis*. *Hosts*: *Perdix daurica*, *Lyrurus tetrix*, *Tetrao urogallus*, *Lagopus mutus* (Phasianidae, Tetraonidae). *Location*: subcutaneous. *Locality*: U.S.S.R. (24).
14. *S. böhmi* (Supperer, 1958) n.comb. Syn. *Ornithofilaria böhmi*. *Host*: *Turdus viscivorus* (Turdidae). *Location*: subcutaneous. *Locality*: Austria (25).
15. *S. gretillati* Chabaud, Anderson and Brygoo, 1959. *Host*: *Agapornis cana* (Psittacidae). *Location*: ? body cavity. *Locality*: Madagascar (10).

Chandlerella Yorke and Maplestone, 1926 (30)

Syn.—*Vagrifilaria* Augustine, 1937; *Parachandlerella* Caballero, 1948.

Amended diagnosis.—Splendidofilariinae. Oesophagus broad, muscular, sometimes clearly divided into anterior, muscular part and longer posterior, glandular part. Junction between oesophagus and intestine clearly demarcated. Cuticle sometimes with bosses. Vulva anterior to end of oesophagus. Caudal papillae often much reduced, generally in rows on ventrolateral surface of caudal extremity. Microfilaria short. Parasites of birds.

A. Oesophagus Club-shaped and Externally Undivided into Muscular and Glandular Parts

1. *C. bosei* (Chandler, 1924) Yorke and Maplestone, 1926 (type). Syn. *Filaria bosei*. *Host*: *Dissemurus paradiseus* (Dicruridae). *Location*: body cavity. *Locality*: India (12,30).
2. *C. travassosi* (Koroliowa, 1926) n.comb. Syn. *Splendidofilaria travassosi*. *Host*: *Merops apiaster* (Coraciidae). *Location*: heart. *Locality*: U.S.S.R. (15).
3. *C. skrjabini* (Petrov and Tschertkowa, 1947) n.comb. Syn. *Cardiofilaria skrjabini*. *Host*: *Hypotriorchis subbuteo* (Falconidae). *Location*: heart. *Locality*: U.S.S.R. (20).

B. Oesophagus Long, Externally Divided into Muscular and Glandular Parts

4. *C. columbigallinae* (Augustine, 1937) n.comb. Syn. *Vagrifilaria columbigallinae*. *Host*: *Columbigallina passerina nigrirostris* (Columbidae). *Location*: body cavity. *Locality*: Virgin Islands (7).
5. *C. periarterialis* (Caballero, 1948) n.comb. Syn. *Parachandlerella periarterialis*. *Host*: *Tyrannus* sp. (Tyrannidae). *Location*: connective tissue of pulmonary arteries. *Locality*: Mexico (8).
6. *C. stantchinskyi* Gilbert, 1932. *Host*: *Oriolus oriolus* (Oriolidae), *Corvus corax* (Corvidae). *Location*: body cavity. *Locality*: U.S.S.R. (13).
7. *C. sinensis* Li, 1933. *Hosts*: *Urocissa sinensis* (Corvidae). *Location*: lung and trachea. *Locality*: China (17).
8. *C. lienalis* Orloff, 1947.* *Host*: *Caccabis chukar* (Phasianidae). *Location*: spleen and splenic vessels. *Locality*: U.S.S.R. (23).
9. *C. braziliensis* Yeh, 1957. *Host*: *Rhamphastus dicolorus* (Ramphastidae). *Location*: cervical air sacs. *Locality*: Brazil (29).
10. *C. singhi* Ali, 1957. *Host*: *Caprimulgus* sp. (Caprimulgidae). *Location*: heart. *Locality*: India (1).

Acknowledgments

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*In Skrjabin and Shikhobolova (23), who give on pages 375, 376 a description of *Chandlerella lienalis* Orloff, 1947, but do not list a paper by this author.

of the genera *Paramicipsella* Chow, 1939 emend. Chabaud and Choquet, 1953 and *Ornithofilaria* Gönnert, 1937. Can. J. Zool. **33**, 107–112 (1955).
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A LACK OF AVIAN AND MAMMALIAN HAEMATOZOA IN THE ANTARCTIC AND CANADIAN ARCTIC[1]

MARSHALL LAIRD

Abstract

Blood films were obtained from 111 animals in New Zealand's Ross Dependency, and 165 on Prince of Wales Island, N.W.T. Most of them were from birds, and all proved negative for haematozoa although not because of any lack of susceptibility. An absence of vectors precludes local transmission in the areas sampled, and while wide-ranging migratory birds nesting in the Arctic could be exposed to infection elsewhere, many of them are normally protected from this hazard by ecological barriers.

Introduction

Penguins have yielded *Plasmodium* in southern waters (6, 9); babesioids and spirochaetes presumably transmitted by the locally abundant tick (*Argas talaje*) = *Ornithodoros capensis* Neumann parasitize *Spheniscus demersus* (L.) on Dassen Island off the South African coast (5); and these birds readily fall victim to such diseases as avian malaria (13) and toxoplasmosis (12) when kept in zoos. Granted an infective reservoir and plentiful vectors, high incidence of haematozoa is obviously inevitable among such susceptible and gregarious birds. However, studies on Macquarie Island (55° S., 160° E.) failed to reveal *Plasmodium relictum* var. *spheniscidae* Fantham and Porter (6), or any other blood parasites, in 121 penguins of four species on this mosquito-free island (10). This removed the suspicion that some vector other than a mosquito might have been responsible for the transmission of a distinctive penguin malaria parasite, and suggested that infections noted in subantarctic birds would prove to be due to transmission in lower latitudes during seasonal dispersal northwards.

Reindeer suffer from tick-borne babesiosis in the Russian Arctic (3), and blood smears from *Rangifer* spp. and northern seals could include *Sarcocystis* spores. *S. richardi* Hadwen was described from *Phoca vitulina* subsp. in subarctic Alaska (7), and the same host is susceptible to *Dirofilaria immitis* Leidy (2). The literature includes earlier records of microfilariae in the blood of captive seals.

Since there were no reports of haematozoa from Antarctica or the Canadian Arctic, the opportunity was gladly taken to examine preparations collected by the Transantarctic (N.Z.) Expedition 1956–58 in New Zealand's Ross Dependency, and by Mrs. Elizabeth Macpherson on Prince of Wales Island, N.W.T.

Materials, Methods, and Results

The animals sampled are listed in Table I. All the penguins were handled at Cape Royds (78° S., 167° E.) on January 4, 1957, blood being drawn from

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beneath a flipper of the living bird. Smears were made from Weddell seals on nearby McMurdo Sound during the same year. A single thin blood film was taken from each of the antarctic animals, two (from the heart or shot wounds) from each of the Canadian ones. The latter were collected during wildlife research in the northern part of Prince of Wales Island (72–74° N., 96–103° W.) between June 3 and September 10, 1958.

After treatment with Giemsa's stain, the slides were scanned for microfilariae under a 16-mm objective. They were then searched systematically for half an hour each, by oil immersion. Blood parasites were not found in any of them.

TABLE I

Study material

Scientific name	Common name	Number examined
	Antarctica	
Pygoscelis adeliae (Hombron & Jacquinot)	Adelie penguin	94
Leptonychotes weddelli (Lesson)	Weddell seal	17
	Canadian Arctic	
Chen hyperborea atlantica Kennard	Snow goose	1
Buteo lagopus s. johannis (Gmelin)	American rough-legged hawk	1
Falco rusticolus obsoletus Gmelin	Gyrfalcon	1
Lagopus lagopus leucopterus Taverner	Willow ptarmigan	12
Lagopus mutus saturatus Salomonsen	Rock ptarmigan	9
Arenaria interpres subsp.	Ruddy turnstone	14
Pluvialis dominica dominica (Müller)	Golden plover	2
Squatarola squatarola (L.)	Black-bellied plover	7
Calidris canutus canutus (L.)	Knot	2
Crocethea alba (Pallas)	Sanderling	4
Erolia bairdii (Coues)	Baird's sandpiper	3
Erolia maritima (Brünnich)	Purple sandpiper	13
Phalaropus fulicarius (L.)	Red phalarope	4
Stercorarius longicaudus Vieillot	Long-tailed jaegar	17
Stercorarius parasiticus (L.)	Parasitic jaegar	2
Larus thayeri Brooks	Thayer s gull	3
Larus hyperboreus hyperboreus Gunnerus	Glaucous gull	6
Sterna paradisaea Pontoppidan	Arctic tern	5
Xema sabini sabini (Sabine)	Sabine's gull	8
Nyctea scandiaca (L.)	Snowy owl	1
Eremophila alpestris hoyti (Bishop)	Horned lark	2
Calcarius lapponicus lapponicus (L.)	Lapland longspur	14
Plectrophenax nivalis nivalis (L.)	Snow bunting	18
Lepus arcticus andersoni Nelson	Arctic hare	7
Dicrostonyx groenlandicus clarus Handley	Varying lemming	1
Lemmus trimucronatus trimucronatus (Richardson)	Brown lemming	1
Alopex lagopus innuitus (Merriam)	Arctic fox	4
Phoca hispida hispida Schreber	Ringed seal	2
Rangifer arcticus pearyi Allen	Barren ground caribou	1

Discussion

Weddell seals and Adelie penguins only rarely leave Antarctica, and the uniformly negative results of this investigation emphasize the absence of biting Diptera and the likelihood that none of the ectoparasites present act as vectors for blood parasites. The latter have never been recorded from any

of the few species of winged birds visiting that continent or breeding there. Indeed, the only such bird belonging to a genus (and for that matter, to an order) including a known host for haematozoa is the arctic tern, a summer visitor.

There were films from only two seals in the collection from Prince of Wales Island, and mammals in general were too poorly represented for profitable discussion. Although babesiosis remains unknown in reindeer or caribou in North America, sarcosporidiosis is common in these animals in subarctic regions. Any statement concerning mammalian babesioids or other ectoparasite-borne haematozoa must await future surveys. On the other hand, there were preparations from 149 birds of 23 species. Their complete lack of haematozoa was in striking contrast to the situation revealed at Kohlmeister Lake (58° 07′ N., 68° 07′ W.), just south of the tree line in Ungava Bay, Quebec, a year later. For of 129 birds of 29 species shot there, 78 (60%) proved to harbor one or more of six genera of blood parasites (original data, unpublished).

Unlike Antarctica, the Canadian Arctic supports mosquitoes. These belong to the subgenus *Ochlerotatus* of *Aedes*, and three species—*A. hexodontus* Dyar, *A. impiger* (Walker), and *A. nigripes* (Zetterstedt)— known to occur farther north than Prince of Wales Island, are quite possibly present there too. Mosquitoes, the only vectors of *Plasmodium*, are represented by several abundant species at Kohlmeister Lake. Even there the only two birds positive for malaria parasites were migrants newly arrived from the south, and no evidence of local transmission was obtained. The situation on Prince of Wales Island appears even less favorable epidemiologically, for Mr. and Mrs. Macpherson (personal communication) noticed no more than a dozen mosquitoes during their entire stay.

Most of the avian infections encountered at Kohlmeister Lake concerned *Leucocytozoon*, *Haemoproteus*, *Trypanosoma*, and microfilariae, in that order. There were high levels of parasitaemia among early spring arrivals, and evidence of local transmission (especially of *Leucocytozoon* and filarioids) was obtained. Ornithophilic simuliids suspected of being vectors of the two latter parasites and perhaps of *Trypanosoma* too (1), and a heleid that probably transmits *Haemoproteus*, were collected in the area.

Of the few birds unlikely to migrate far enough southwards for transmission to be a possibility during the northern winter or on the return flight, only the two species of ptarmigan were adequately sampled. Their freedom from blood parasites contrasted sharply with the occurrence of four haematozoan genera and a 100% incidence of *Leucocytozoon* in seven willow ptarmigan shot at Kohlmeister Lake. The apparent absence of transmission on Prince of Wales Island is supported by entomological data. Not only are mosquitoes very scarce there, but the only possibly ornithophilic simuliid of the arctic islands, *Cnephia borealis* (Mall.), is not believed to occur so far north, and vertebrate-biting heleids are unknown beyond the tree line (information supplied by Entomology Research Institute, Ottawa).

The dozen or so species of birds that can be expected in the Canadian Arctic in winter (14) include not only ptarmigan but three others listed in

Table I, the American rough-legged hawk, gyrfalcon, and snowy owl. Migrating as they do far to the south, it might be wondered why none of the remaining 18 species harbored haematozoa acquired elsewhere. However, 14 of them, comprising 90 (60%) of the 149 birds handled, are referable to the Charadriiformes, the dominant avian order in the Arctic (14). Earlier authors (e.g. 16) have commented on the absence of haematozoa from members of this order in areas where other birds harbor a variety of these parasites. Their relative freedom from infection was strikingly apparent at Kohlmeister Lake, where only one (3%) of the 35 Charadriiformes examined (a black-bellied plover harboring *Leucocytozoon*) was parasitized, compared with 77 (82%) of the other 94 birds collected.

This reflects no special immunity, for besides the record just mentioned there are scattered reports of blood parasites from diverse members of the order. Thus *Leucocytozoon* has been found in a sandpiper in Ontario (1), Wenyon (15) quoted several records of *Haemoproteus* from gulls and snipe, and *Plasmodium* is known from the dowitcher (8) and a tern (4) in the U.S.A. and from a gull in Australia (11). It is submitted that the explanation is an ecological one, Charadriiformes being typically based on tundra, open uplands, and exposed shores and river flats where exposure to biting Diptera must be very much less than that suffered by birds of the forest, scrub, and savannah.

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Contents

Page

M. A. Ali, W. R. Stevenson, and Judith S. Press—Histophysiological studies on the juvenile Atlantic salmon (*Salmo salar*) retina. I. Rates of light- and dark-adaptation 123

Clare Bradley—The effect of certain chemicals on the response to electrical stimulation and the spontaneous rhythmical activity of larvae of *Phocanema decipiens* 129

S. P. Gupta—The effects of ultraviolet radiation on the infective stages of *Trichostrongylus retortaeformis* Zeder (Nematoda) 137

K. C. Sanwal—A key to the species of the nematode genus *Aphelenchoides* Fischer, 1894 143

Marshall Laird—Distomiasis in Tokelau islanders 149

Donald Fairbairn—The in vitro hatching of *Ascaris lumbricoides* eggs 153

L. Y. Wu—*Paratylenchus tenuicaudatus* n. sp. (Nematoda:Criconematidae) . 163

K. A. Wright—Observations on the life cycle of *Capillaria hepatica* (Bancroft, 1893) with a description of the adult 167

B. E. Hooper—Marine nematodes from the coast line of the Gulf of Mexico . 183

Roy C. Anderson—*Splendidofilaria wehri* n. sp. with a revision of *Splendidofilaria* and related genera 201

Marshall Laird—A lack of avian and mammalian haematozoa in the Antarctic and Canadian Arctic 209